

68 fo 1

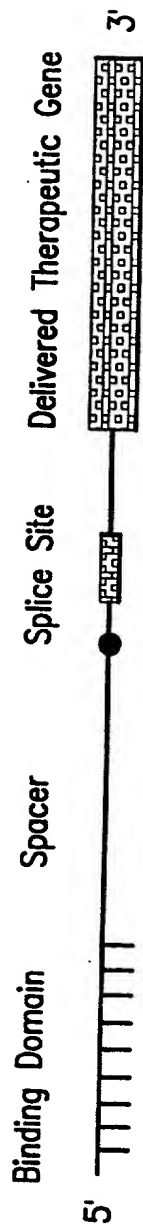


FIG.1A

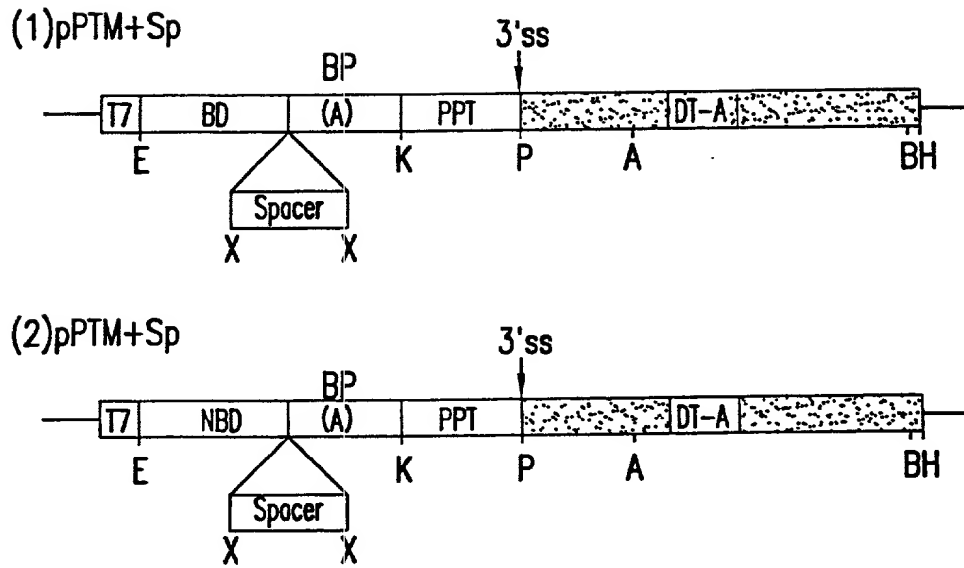


FIG.1B

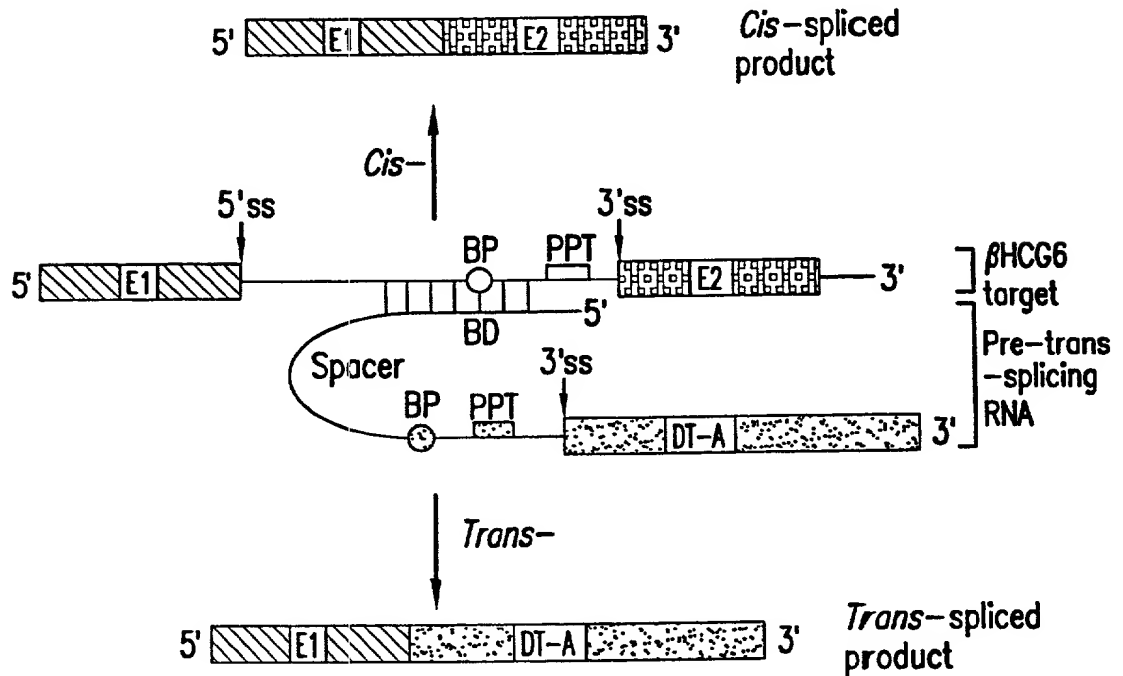


FIG.1C

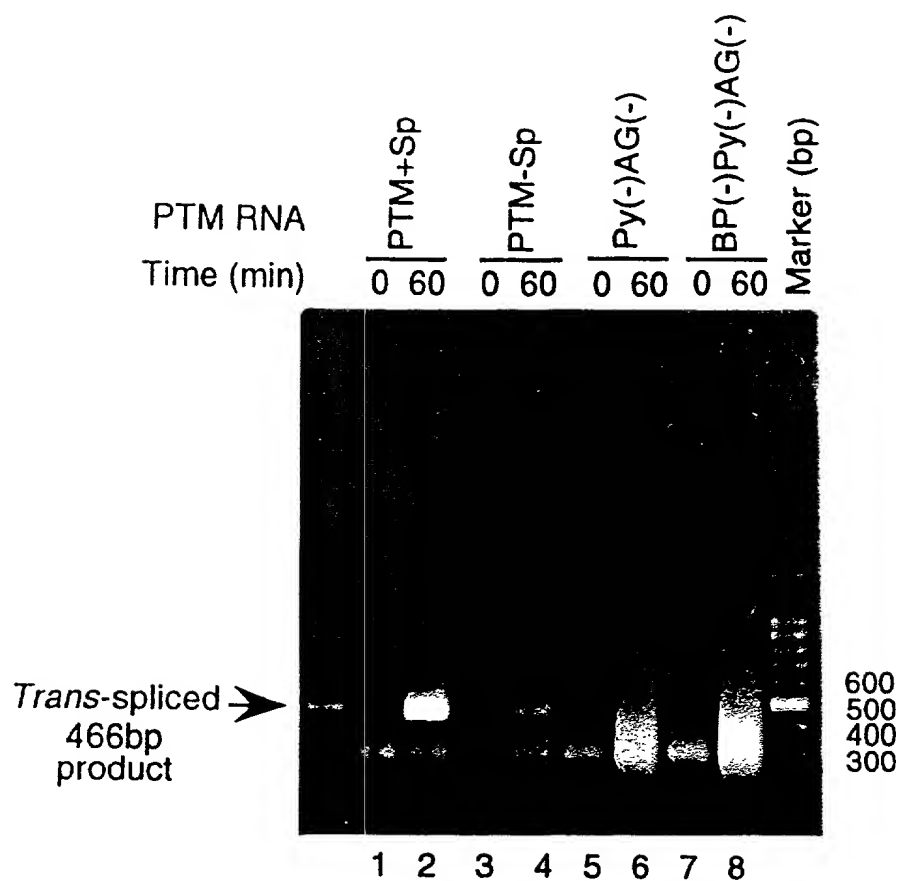


FIG.2A

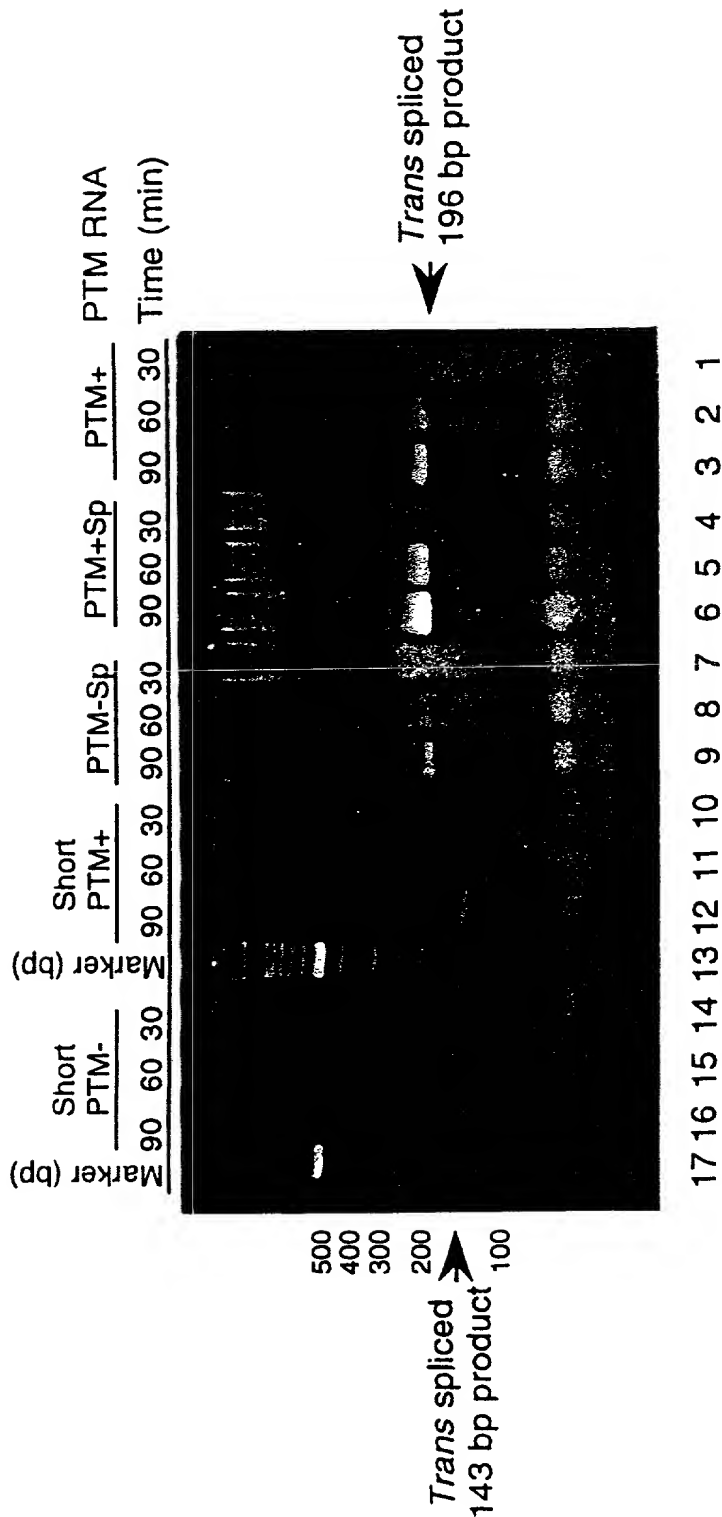
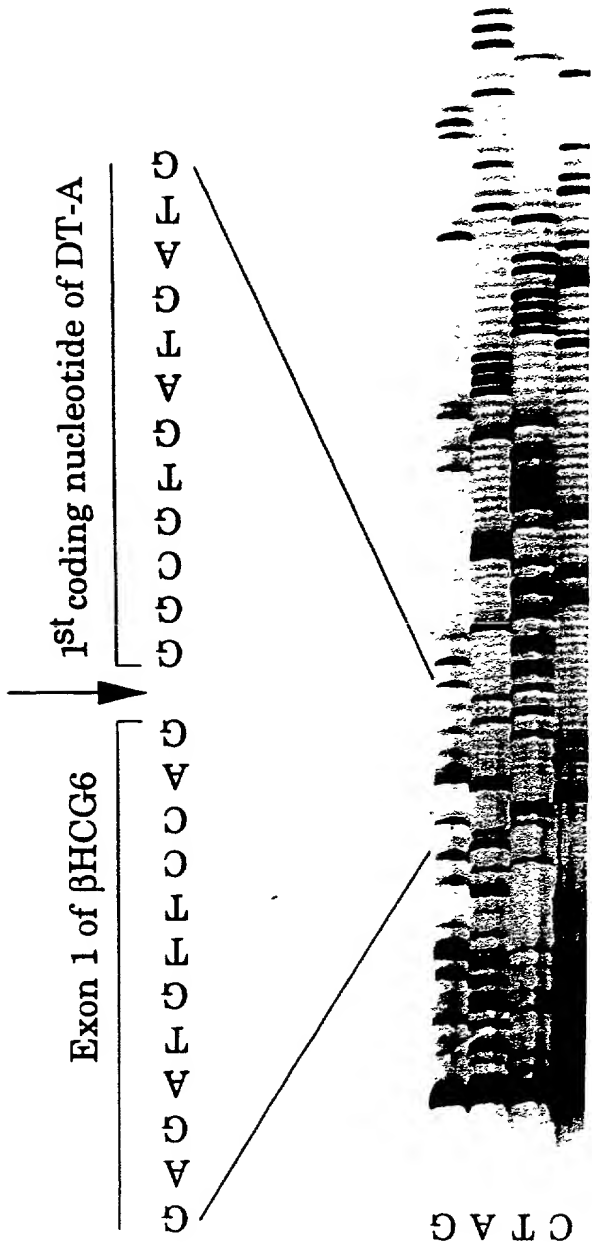


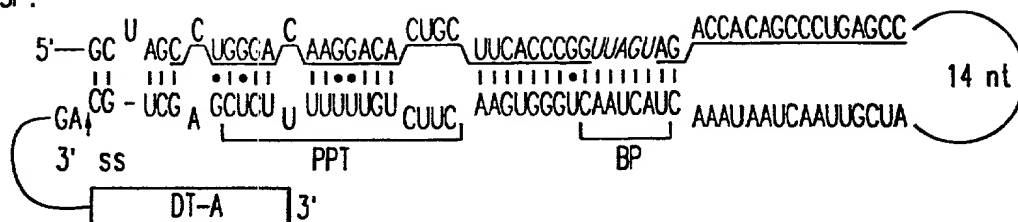
FIG.2B



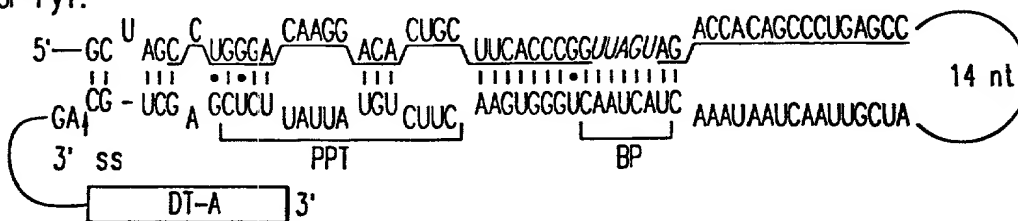
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## 1. PTM+SF:



## 2. PTM+SF-Py1:



## 3. PTM+SF-Py2:

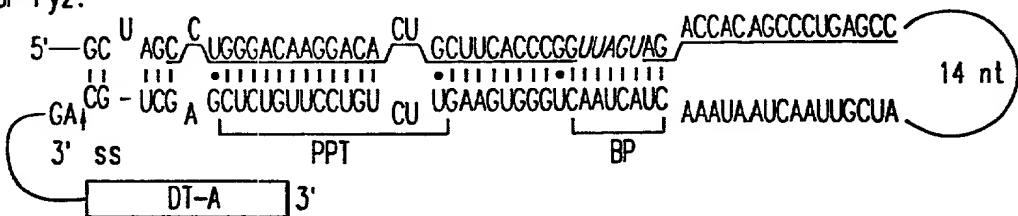


FIG.4A

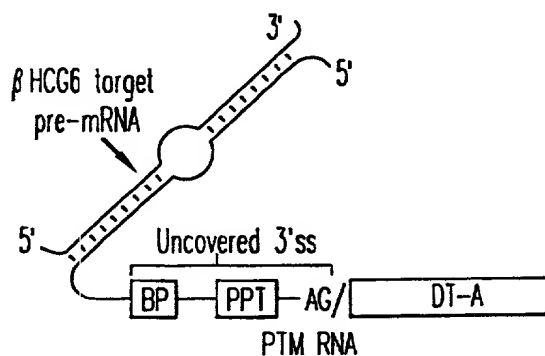


FIG.4B

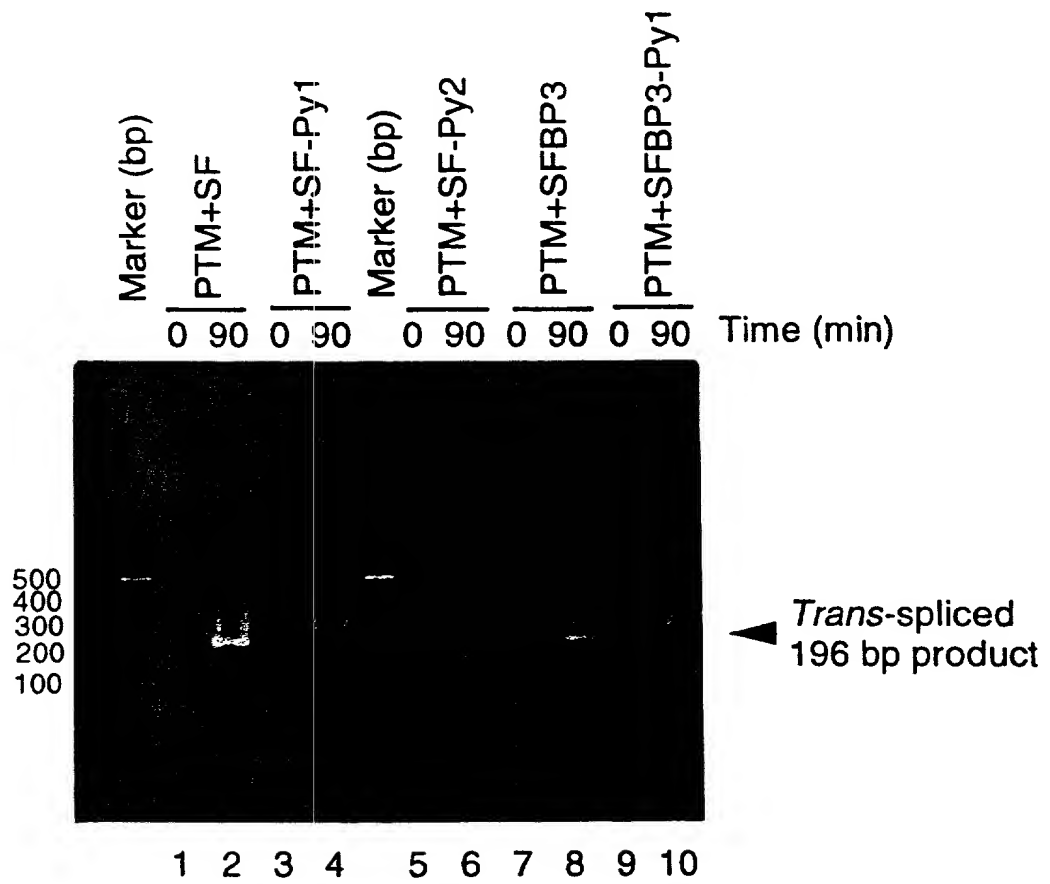


FIG.4C

8 of 8

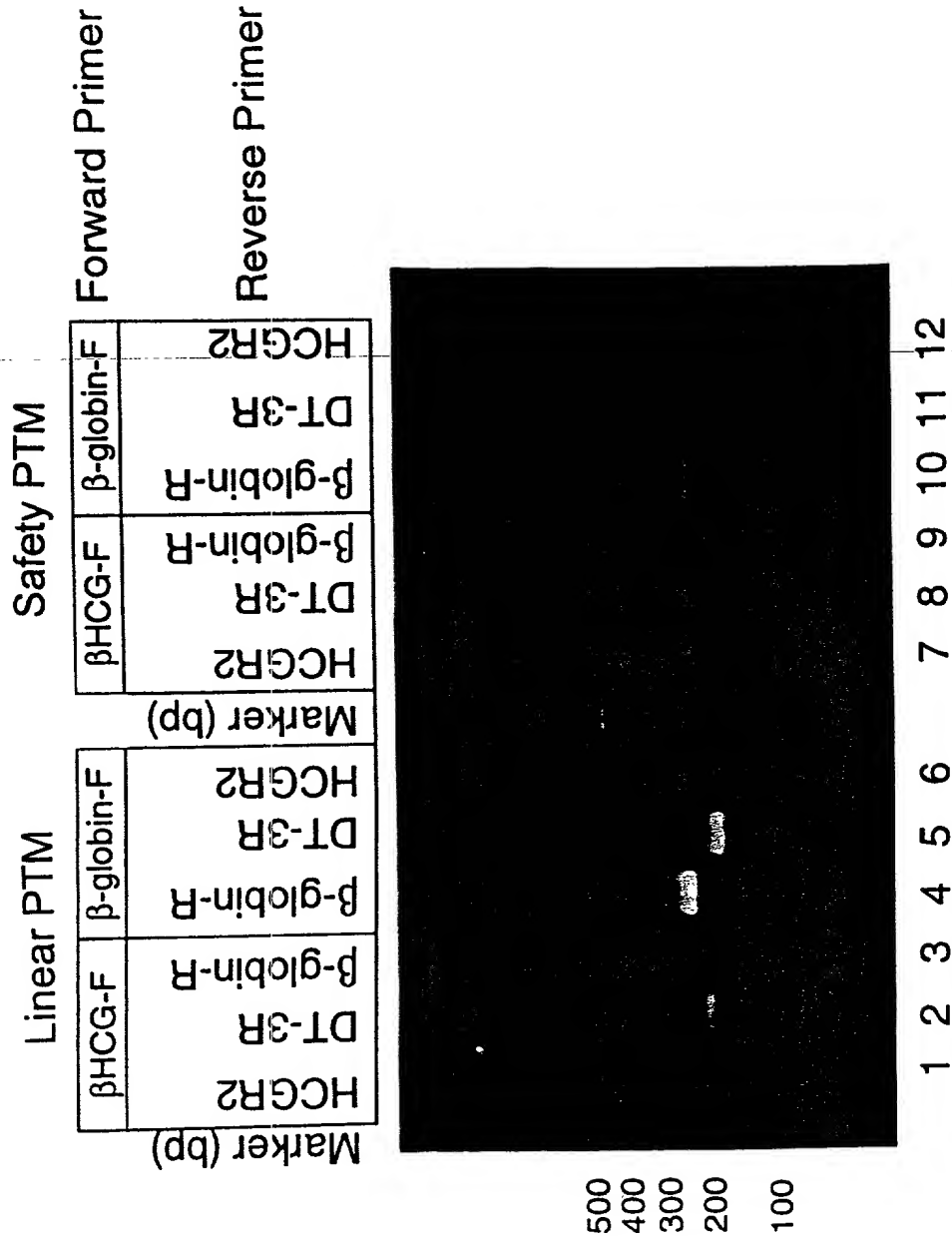


FIG.5

68 fo b

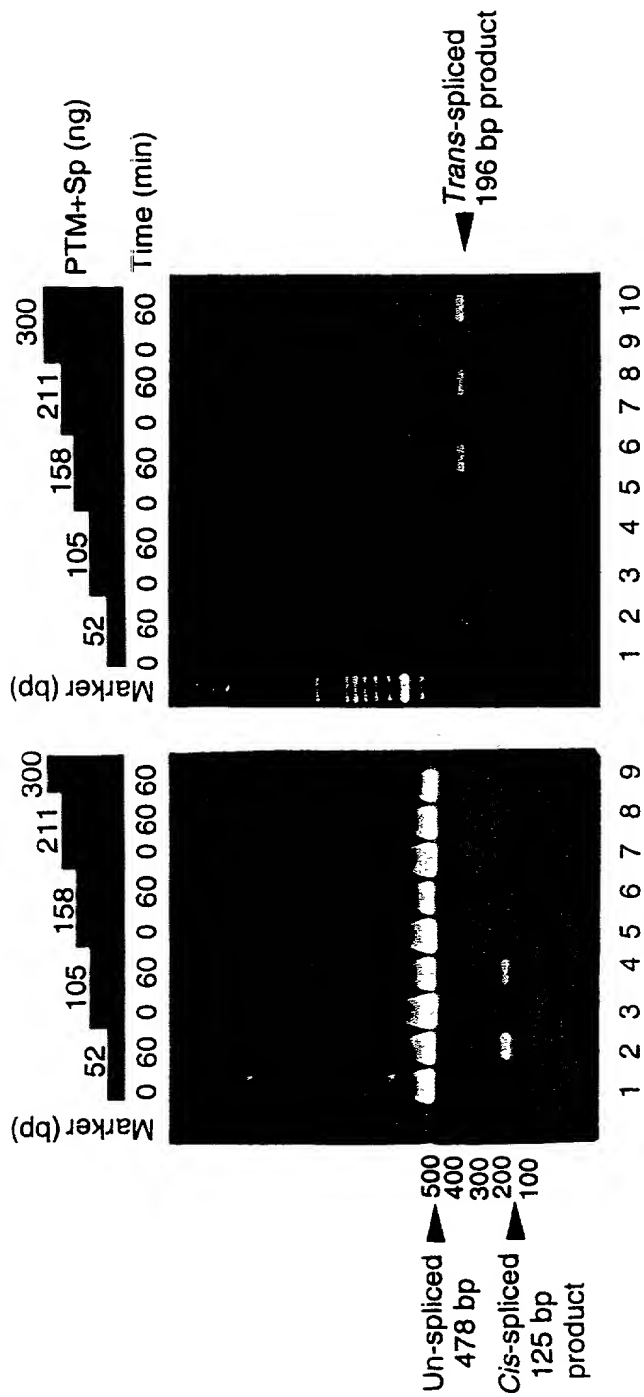


FIG. 6B

FIG. 6A

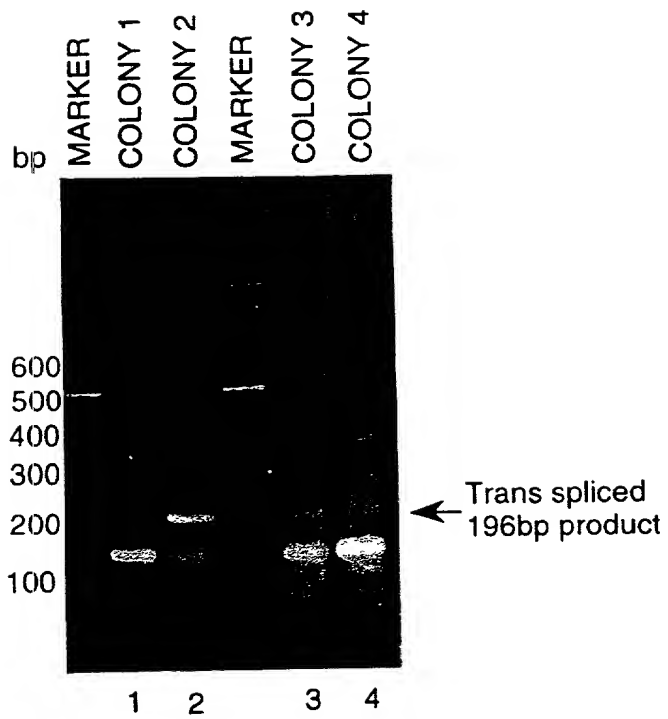


FIG.7A

68 2 11

EXON 1 OF  $\beta$ HCG6 ↓  
 5'-CAGGGACGCACCAAGGATGGAGATGTTCCAG-GCGCTGATGATGTTGTT  
 ↓ 1ST CODING NUCLEOTIDE OF DT-A  
 GATTCTTCTTAAATCTTTGTGATCGAAACCTTTCTTCGTACCAACGGGACTA  
 AACCTGGTTAIGTAGATTCCATTCAAAAA-3'

FIG.7B

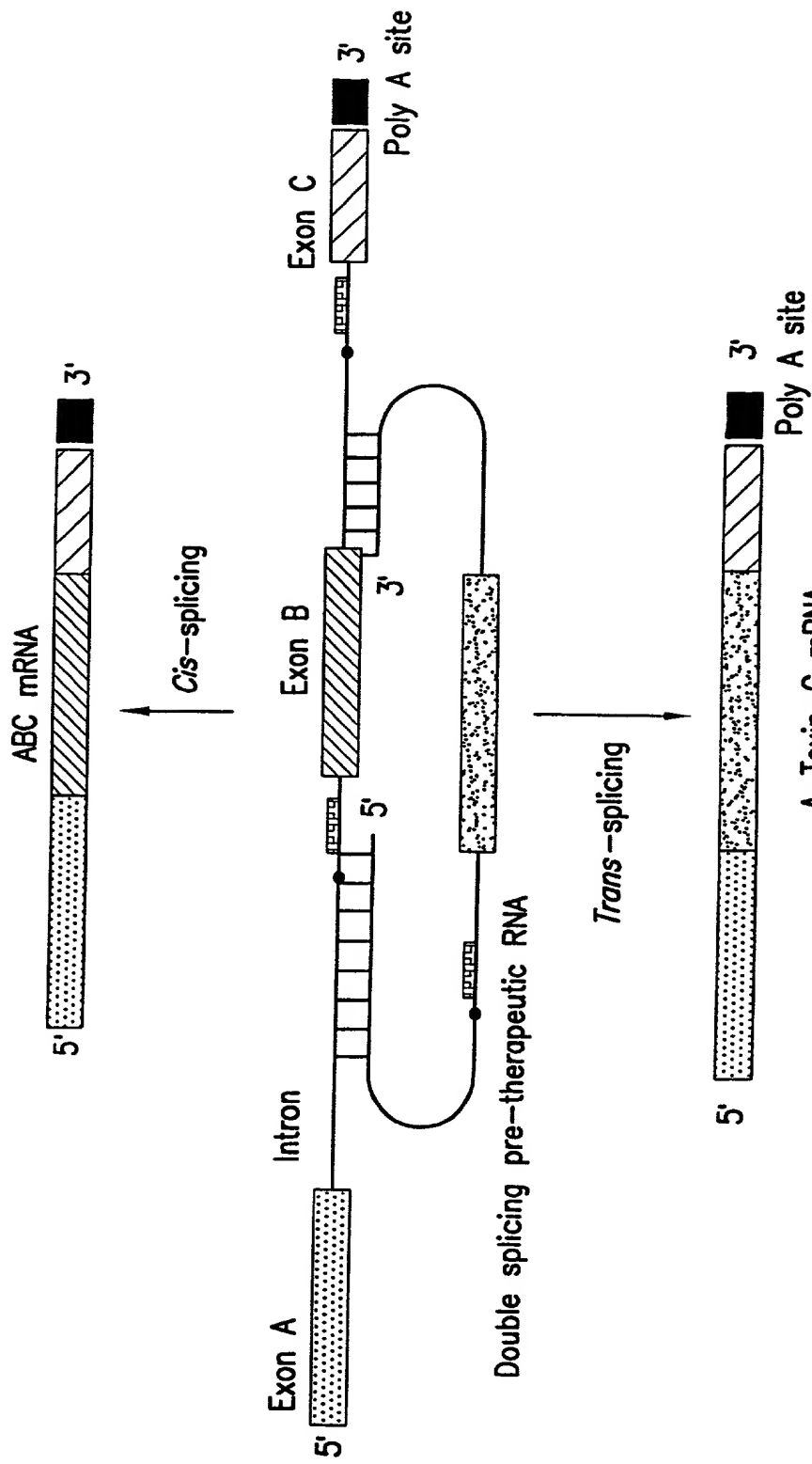
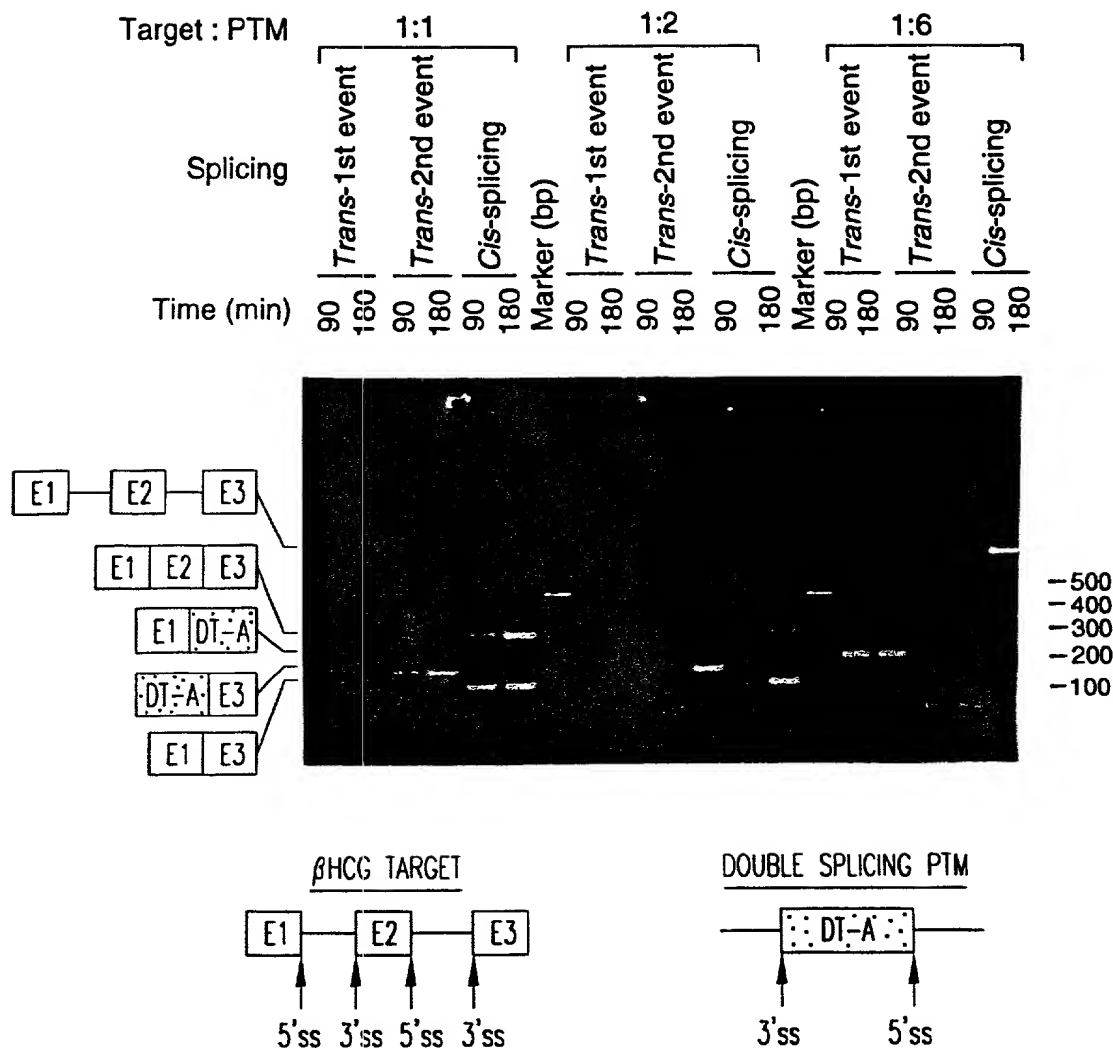


FIG.8A



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#### Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

#### Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

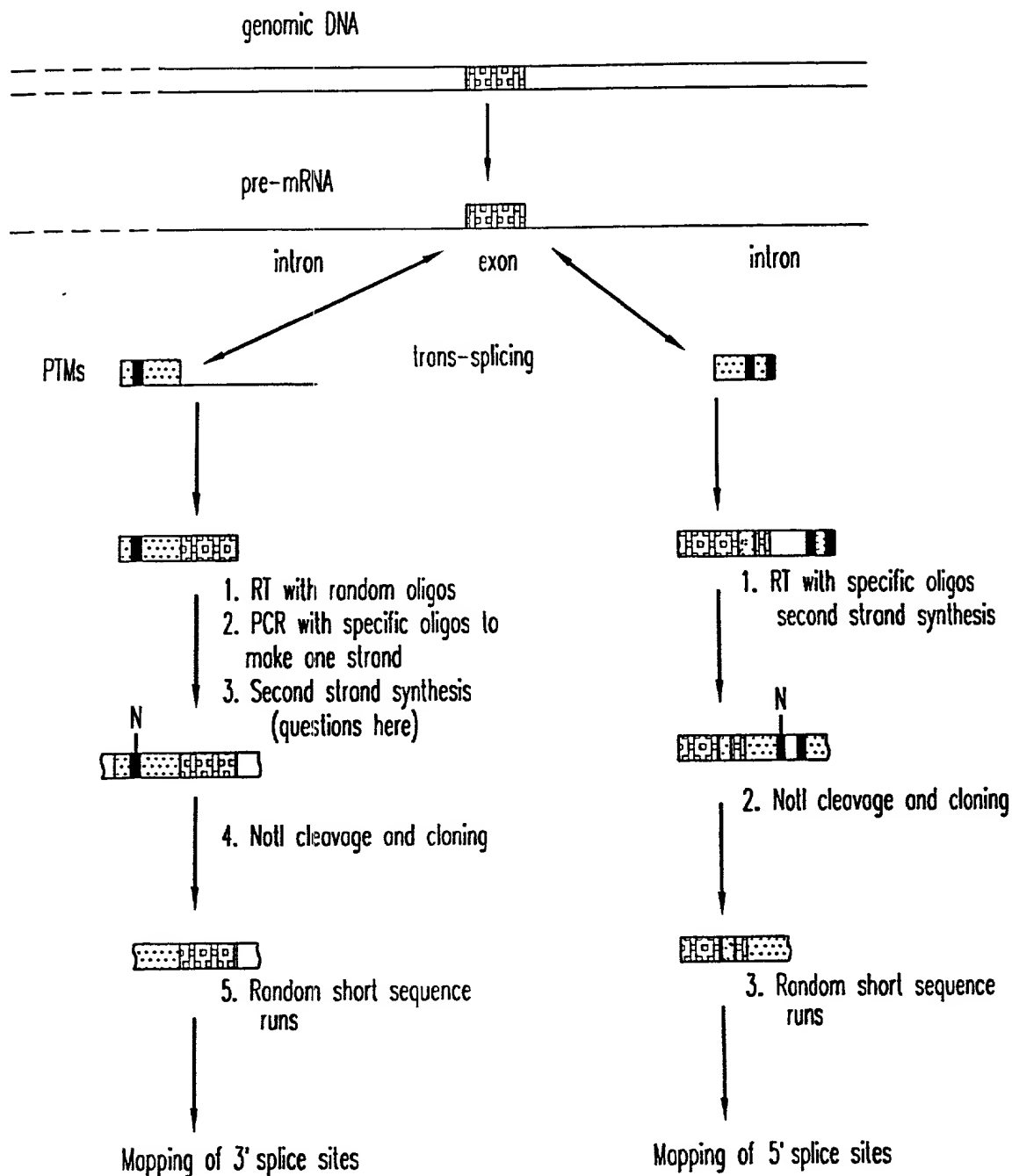


FIG.9

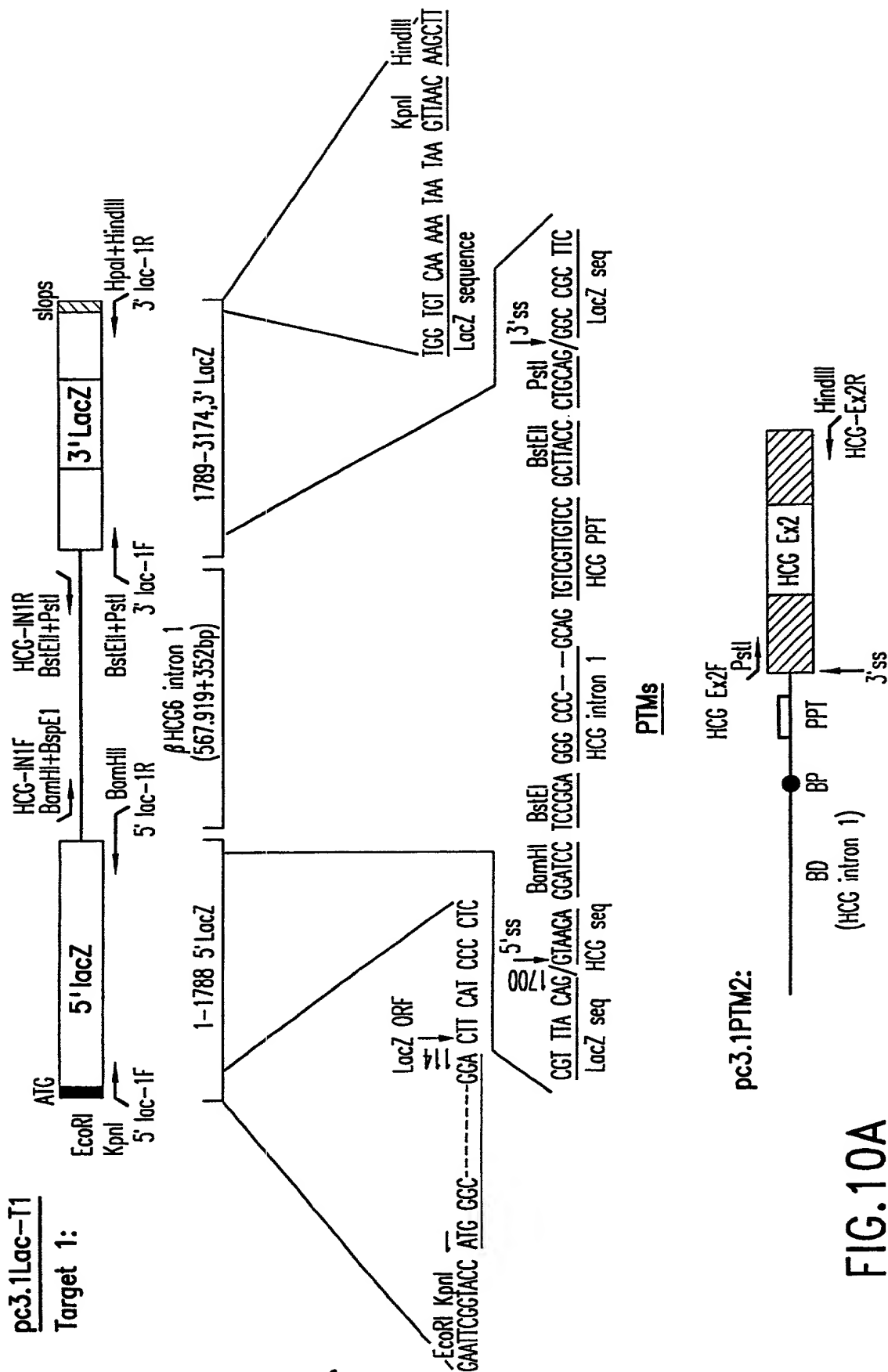


FIG. 10A

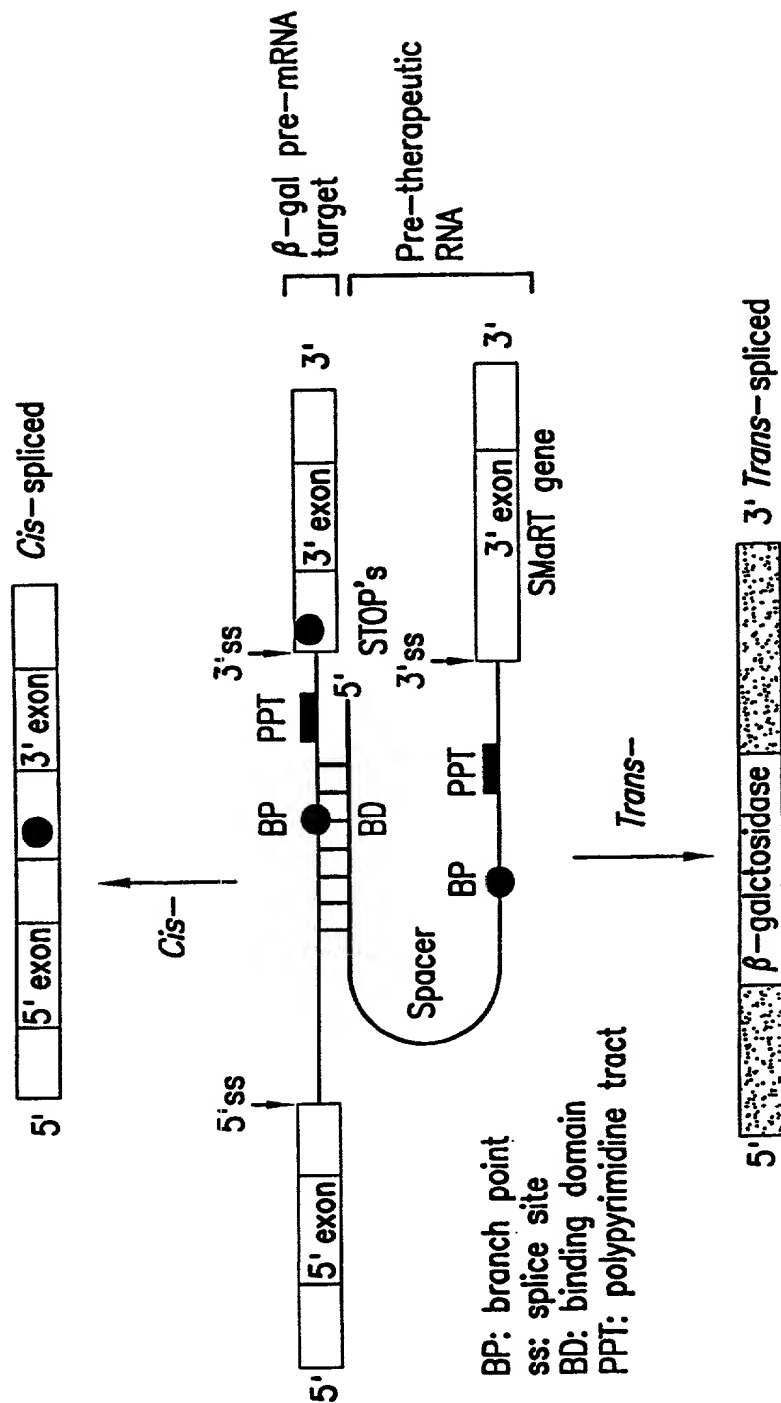


FIG. 10B

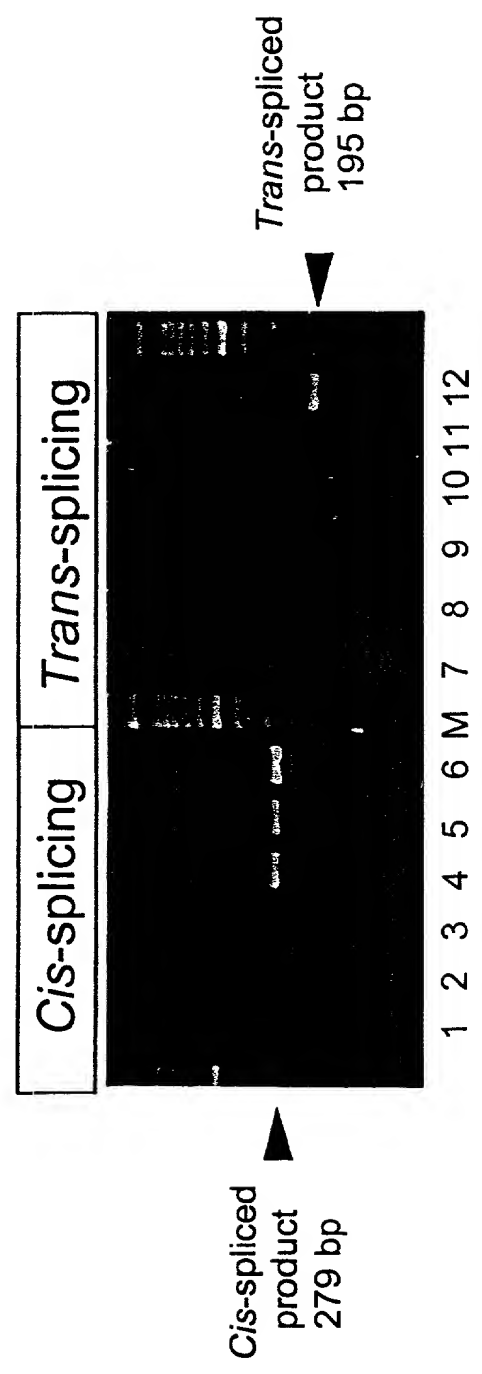
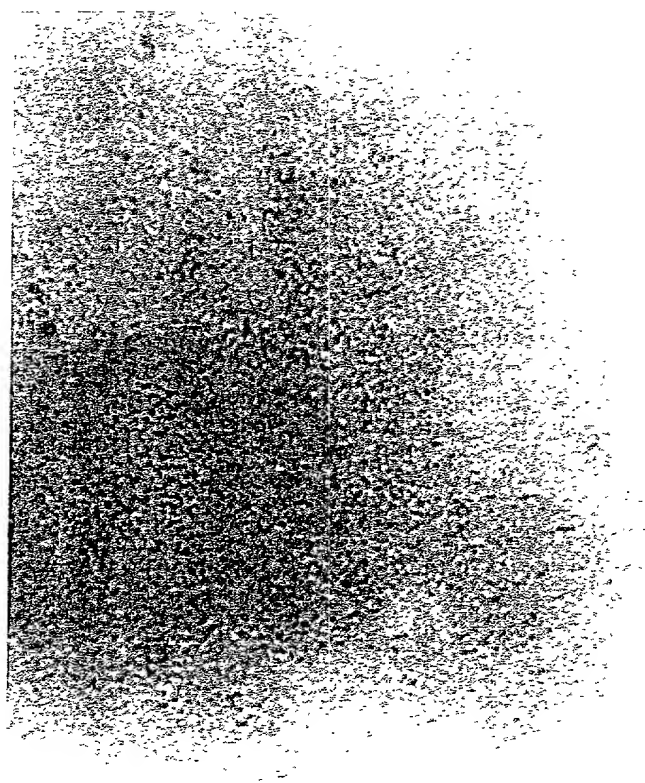


FIG. 11A

[illegible]

•

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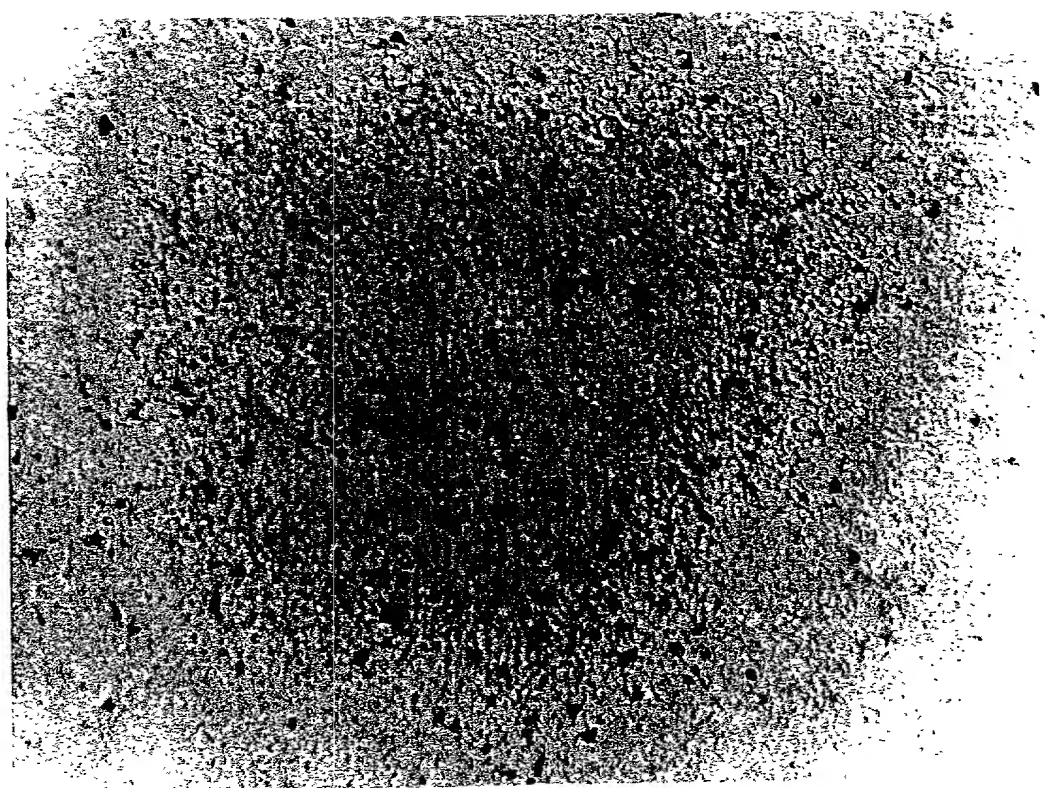


FIG.11C

09941492-082901

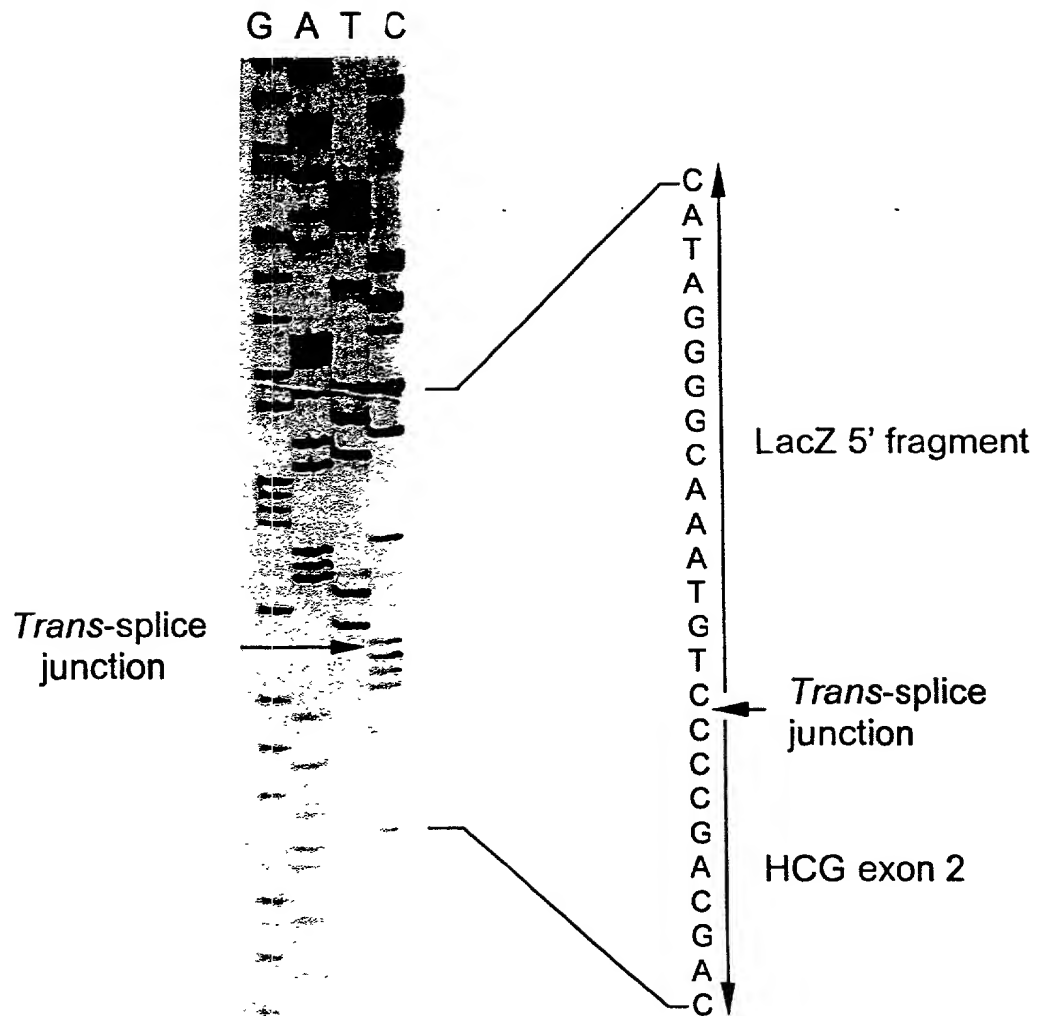


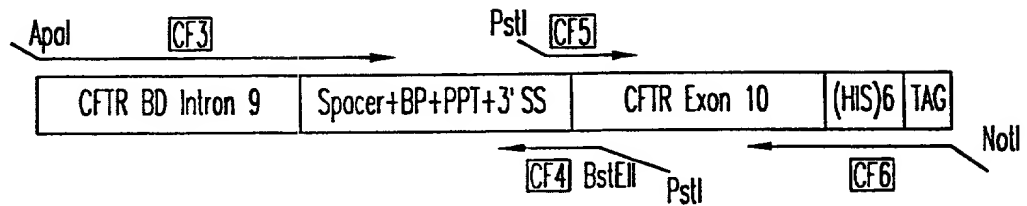
FIG.12A



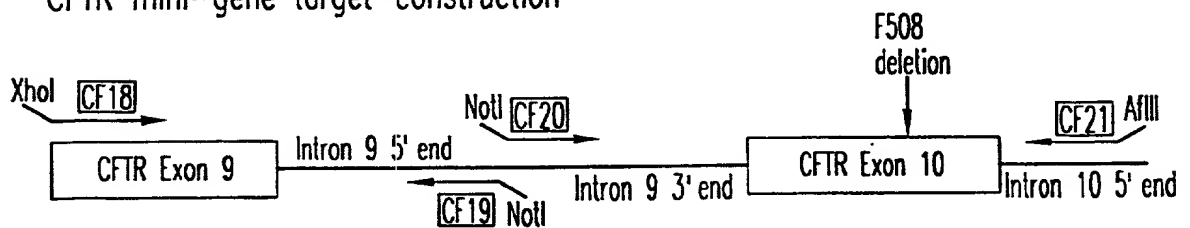


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CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

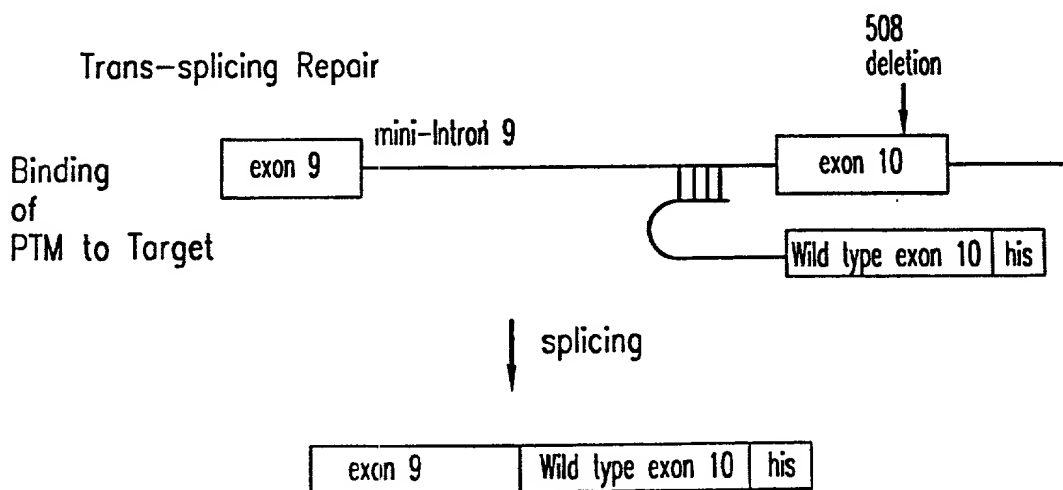


FIG.13

Downloaded from www.ashg.org

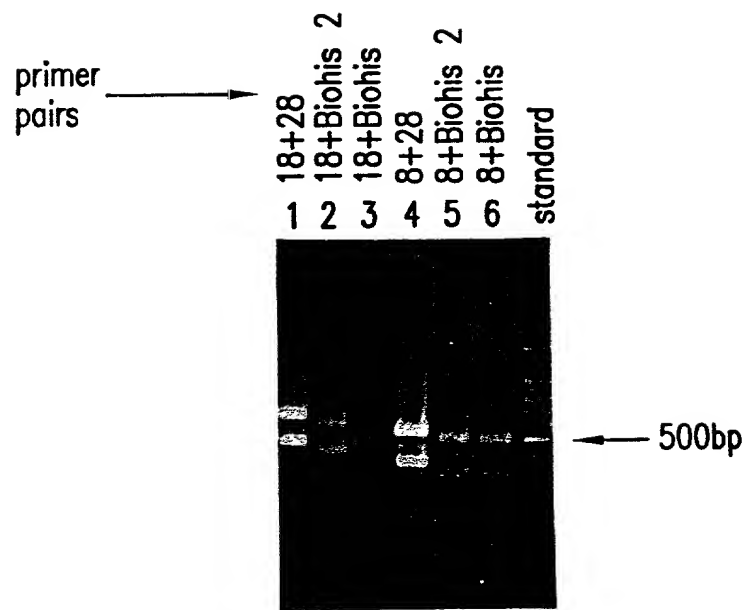
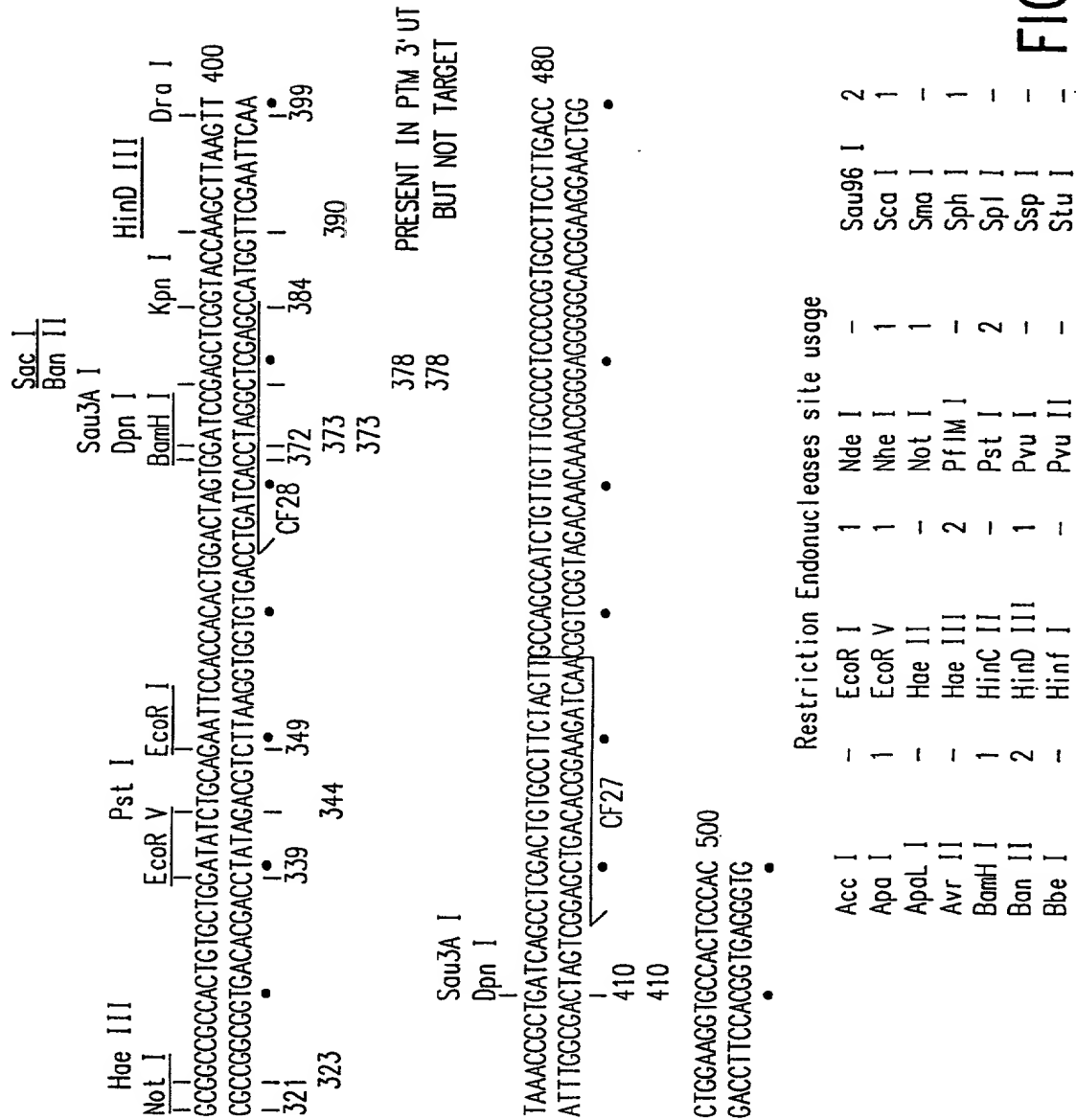


FIG.14

### Positions of Restriction Endonucleases sites (unique sites underlined)

**FIG. 15A**





+

F508 deletion

CFTR Target  
(mini-gene)



Mini-intron 9  
(~0.6kb)

Cotransfect PTM and target molecules in HEK 293 cells  
and detect repaired CFTR mRNA by RT-PCR.

Repaired  
CFTR mRNA

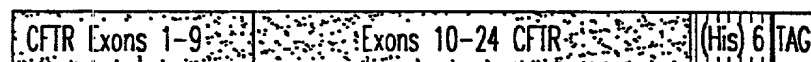


FIG.16

Double Splicing  
PTM

CFTR BD intron 9	Spacer+BP+PPT+3'SS	CFTR exon 10	Spacer+BP+PPT+5'SS	CFTR BD intron 10
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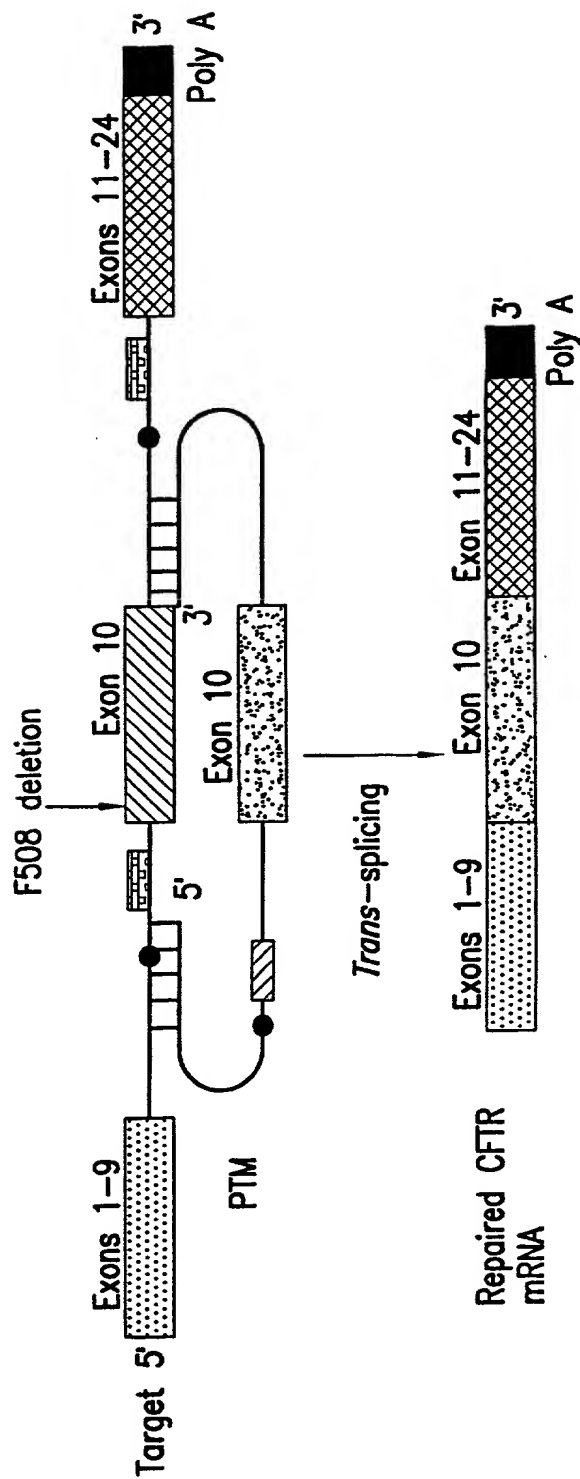


FIG.17

# Double Trans-splicing Specific Target

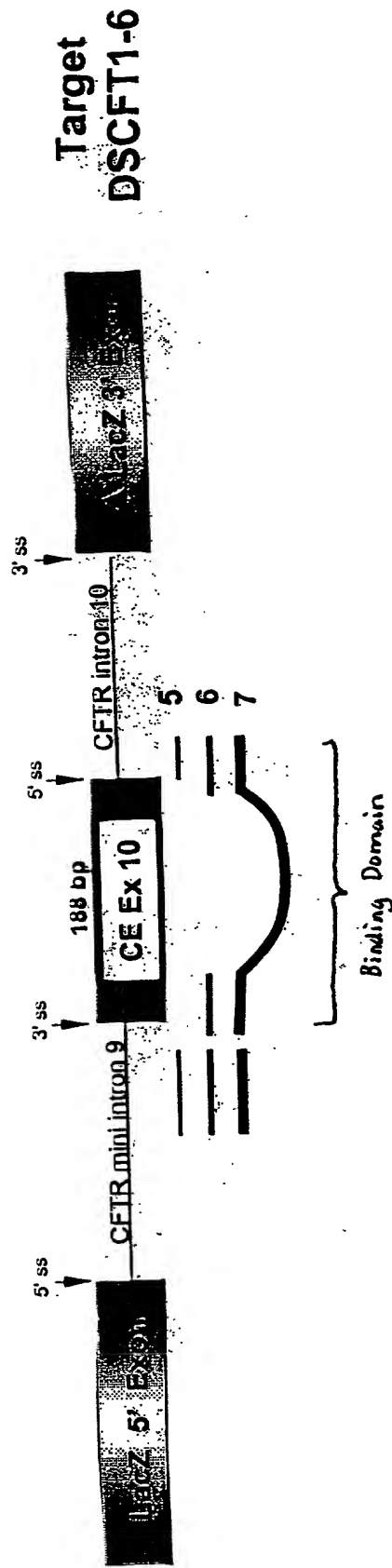
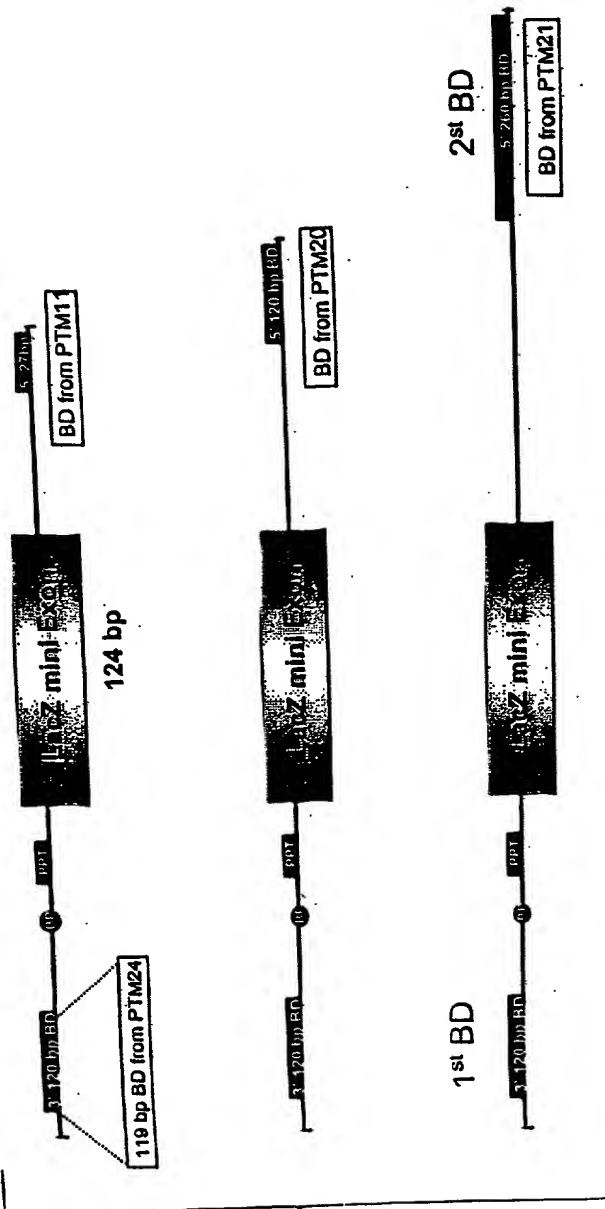


Figure 18

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Double Splicing PTMs



**DSPTM-5**

PTM with 27 bp BD & masks 5' single splice site

**DSPTM-6**

PTM with 120 bp BD & masks both 5' & 3' splice sites

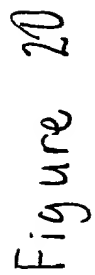
**DSPTM-7**

PTM with 260 bp BD masking both the ss & the entire CFTR Ex10

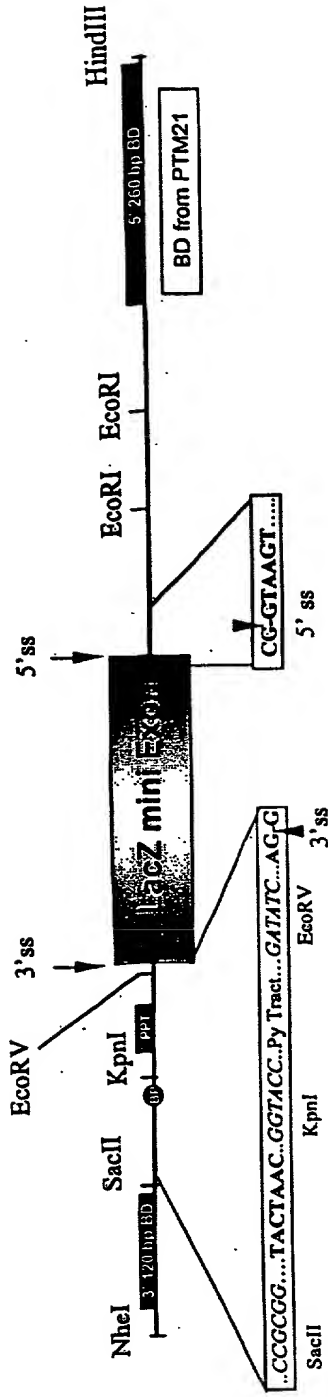
Figure 19

**Double Trans-splicing PTMs**

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# **Important Structural Elements of DSPTM-7:** (Double splicing PTM with all the necessary splice elements i.e. has both 3' and 5' functional splice sites and the binding domains)



**(1) 3' BD (120 BP) :** GATTCACCTTGCTCCAAATTATCATCTCAAGCAGAAGTGATATCTTATTGTAAAGATTCTTAACTCATTTGATTC  
AAATATTTAAATACTCTCTGTTTCATACTCTGCTATGCAC

**(2) Spacer sequences (24 bp):** AACATTATTATAACGTTGCTCGAA

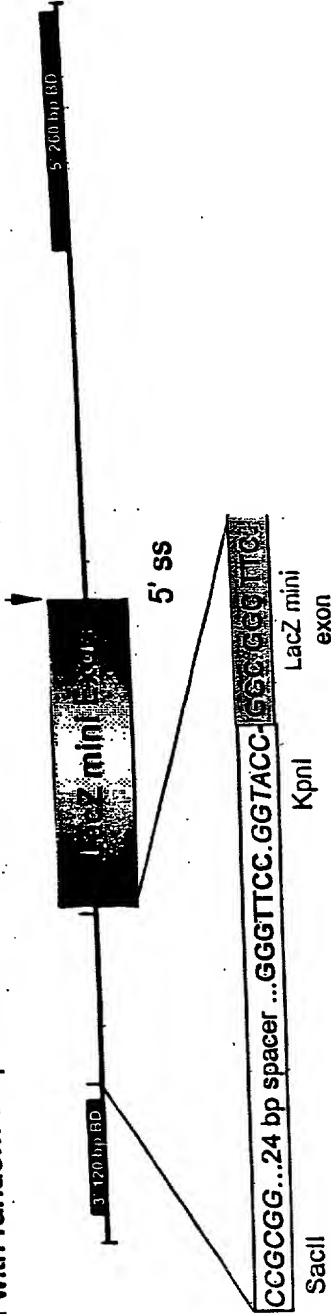
**(3) Branch point, pyrimidine tract and acceptor splice site:** TACTAAC T GGTACC TCTCTTTTTTTTTT GATA TC CTGCAG **LacZ mini exon**  
3' ss EcoRV PPT KpnI BP

**(4) 5' donor site and 2<sup>nd</sup> spacer sequence:** **LacZ mini exon** GTAAGT GTTATCACCGATATGTCTAACCTGATTCGGGCCCTTCGATACG  
CTAAGATCCACCGG

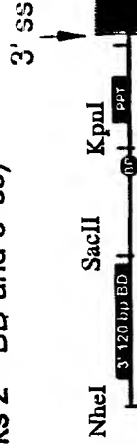
**(5) 5' BD (260 BP) :** TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATTCATTGGAA  
ACACCAATGATTTTCTTTAATGGTCCCTGGCATAATCCTGGAAACTGATAACACAAATGAAATTTCTTCCACTGTGCTTAA  
AAAAACCCCTCTGAAATTCCTCCATTTCTCCCATATATCATCATTAACAACTGAACCTCTGGAAATAAAACCCATCATTTAATACTCA  
TTATCAAAATCACGC

Figure 21

**DSPTM8** : (▲ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



**PTM29** (lacks 2<sup>nd</sup> BD and 5' ss)



**PTM30** (lacks 1<sup>st</sup> BD and 3' ss)



Figure 22

Mutants



# Double Trans-splicing Produces Full-length Protein



β-gal →  
(120 kDa)

1 2 3 4 5 6 7

Lane 1: DSCFT1.6 Target alone 25 μg  
 Lane 2: DSPTM7 25 μg  
 Lane 3 Target + PTM #6 25 μg  
 Lane 4: Target + PTM #9 25 μg  
 Lane 5: Delta 3' splice mutant alone 25 μg  
 Lane 6: Target + Delta 3' ss 25 μg  
 Lane 7: Target+PTM29+30 (mutants) 25 μg

Figure 24

# Restoration of $\beta$ -Gal Function by Double Trans-splicing

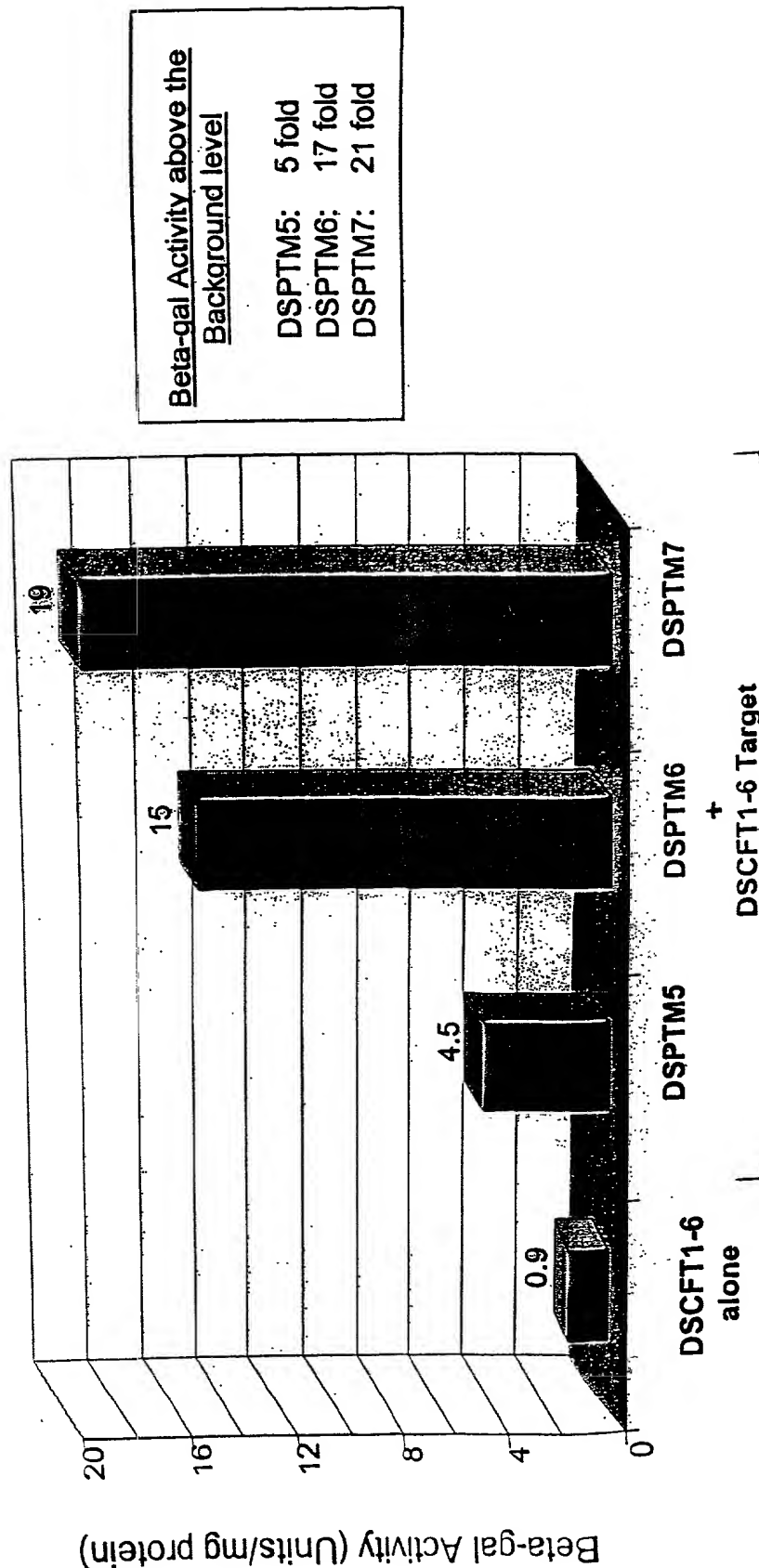


Figure 25

# Restoration of $\beta$ -gal activity is due to double RNA trans-splicing events

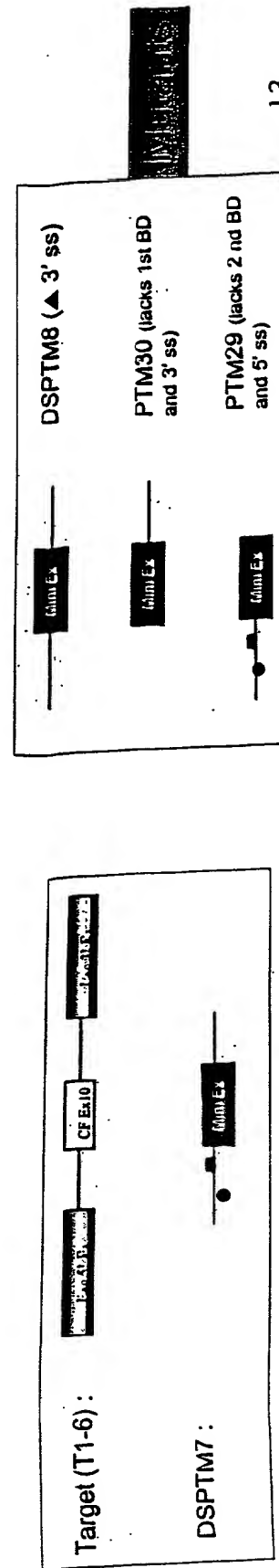
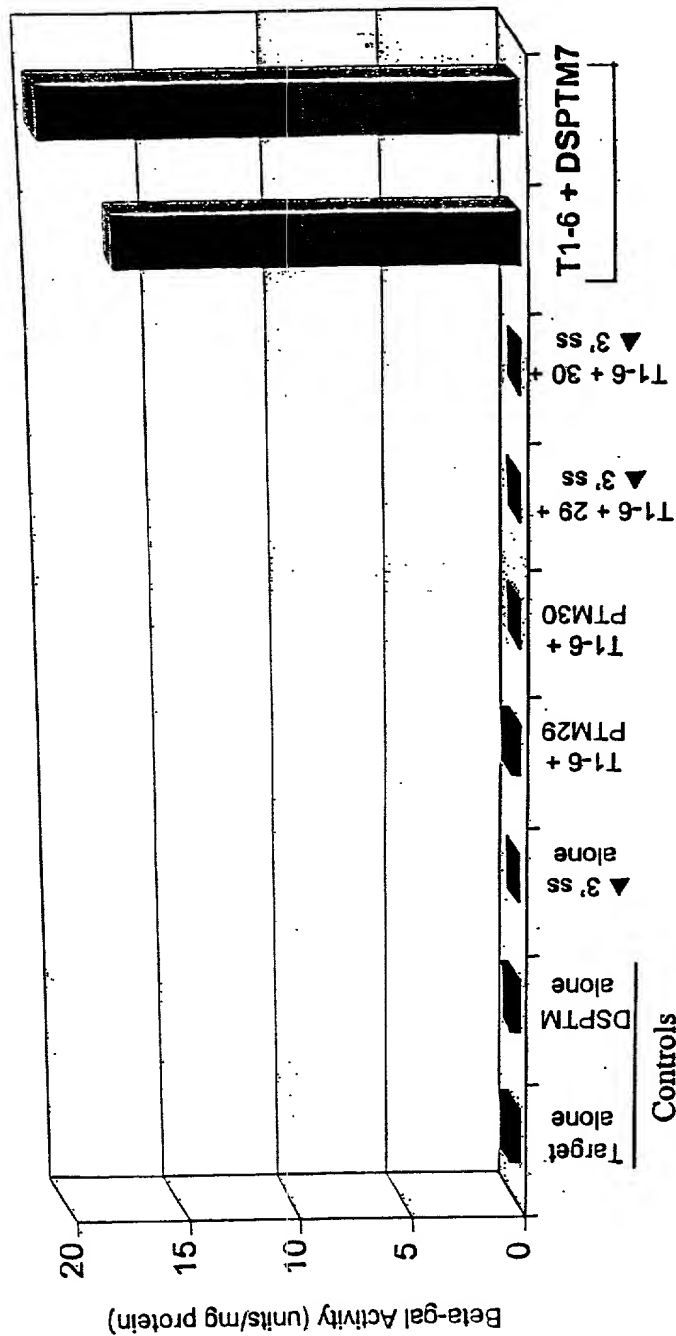


Figure 26



# Double Trans-splicing: Titration of Target & PTM

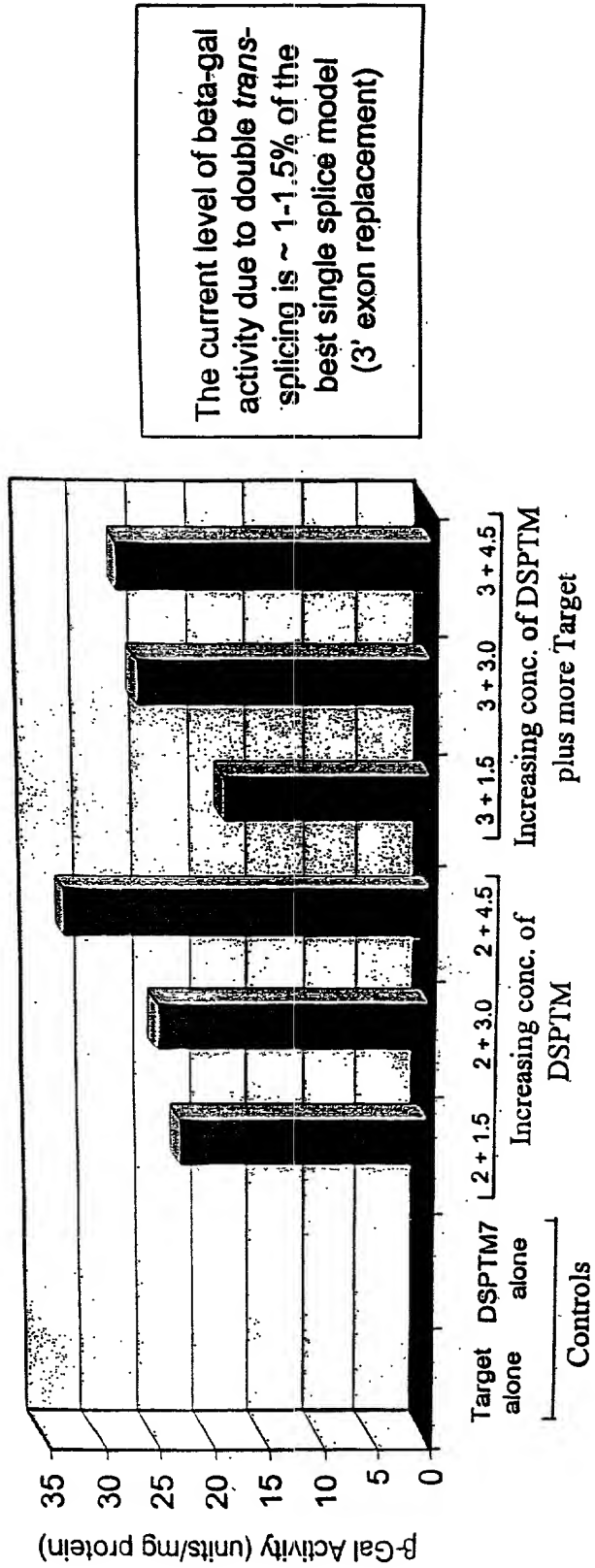
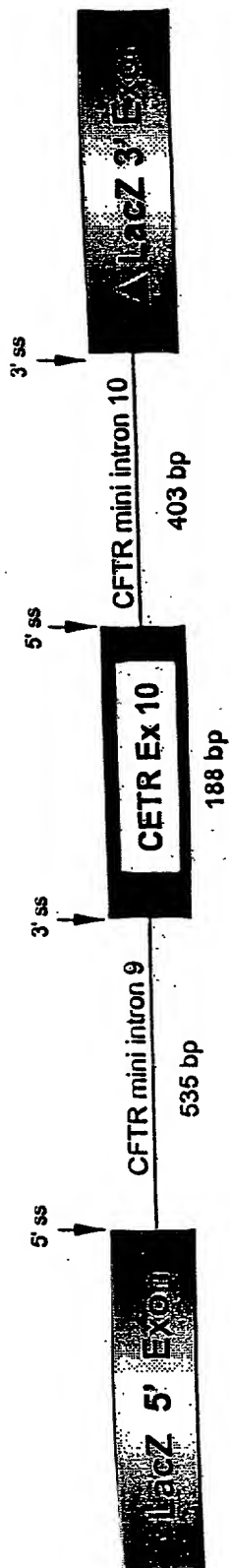


Figure 27

**DSCFT1-6 (Specific Target):**



**DSHCGT1 (Non-specific Target):**

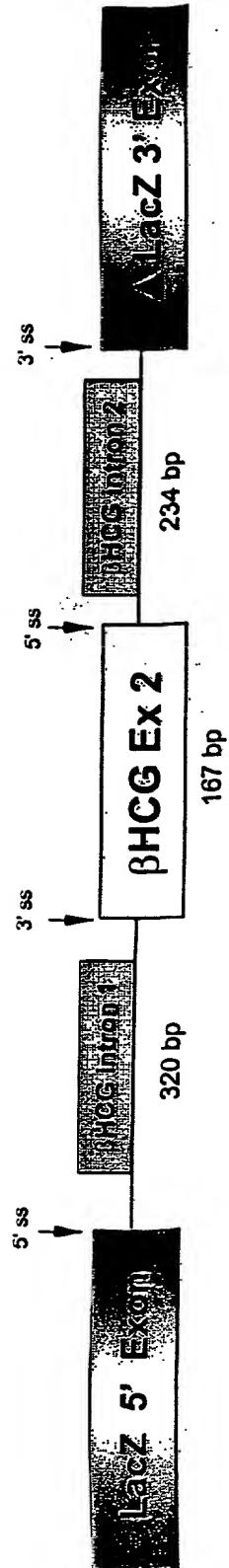


Figure 28

# Specificity of double *trans*-splicing Reaction

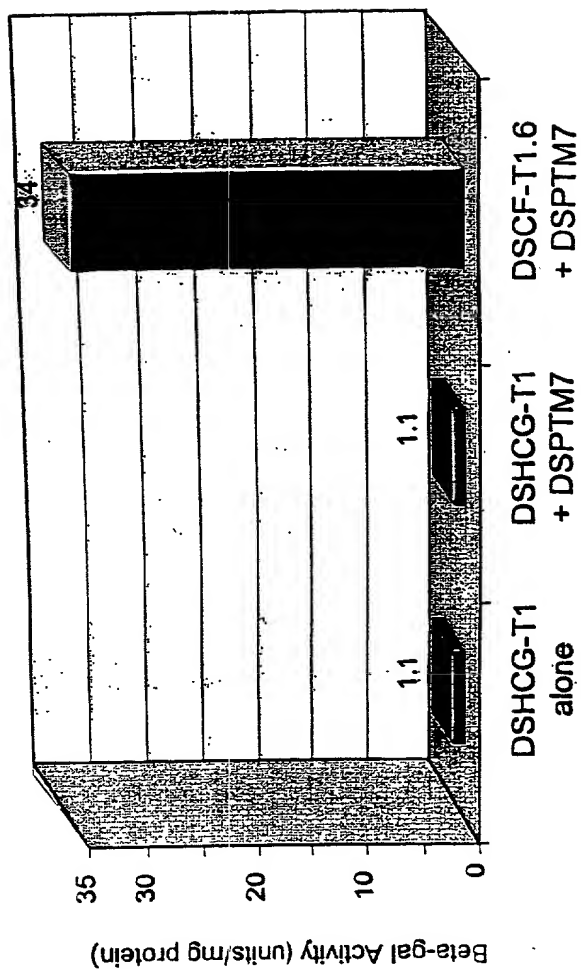


Figure 29

Replacement of a single Intron and Intron  
 Some transgenic plants are known to have a single Intron and Intron

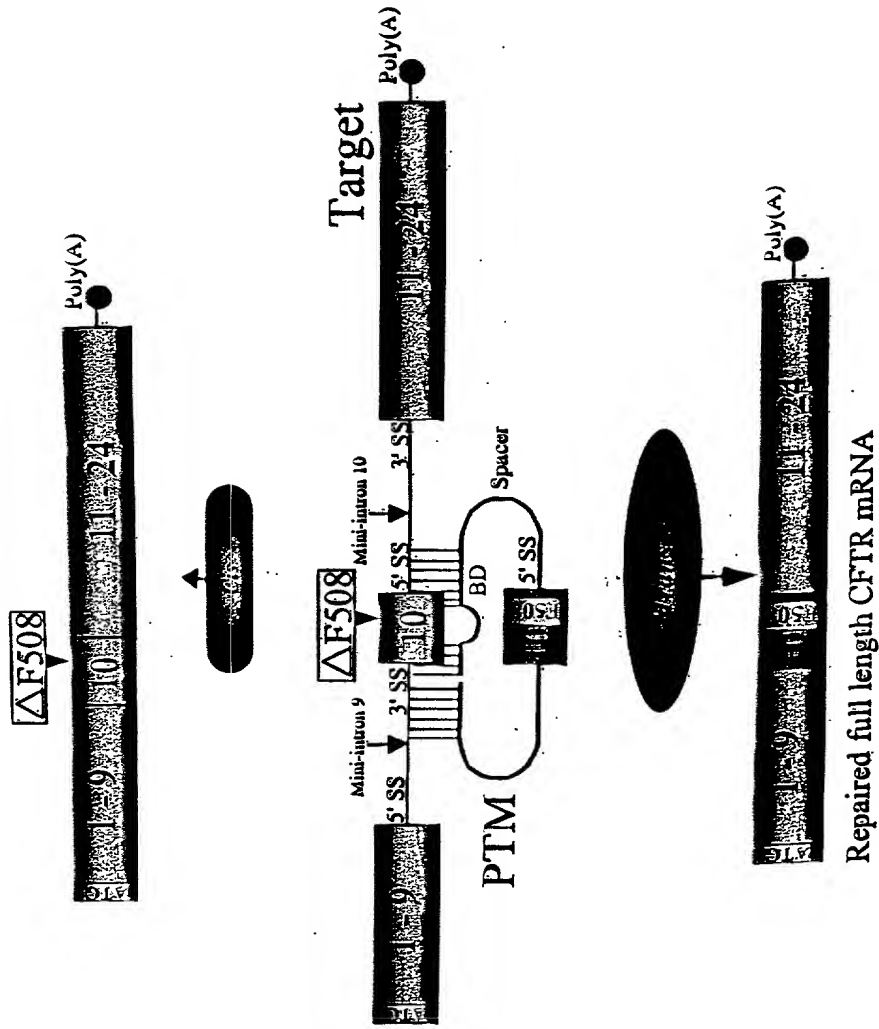
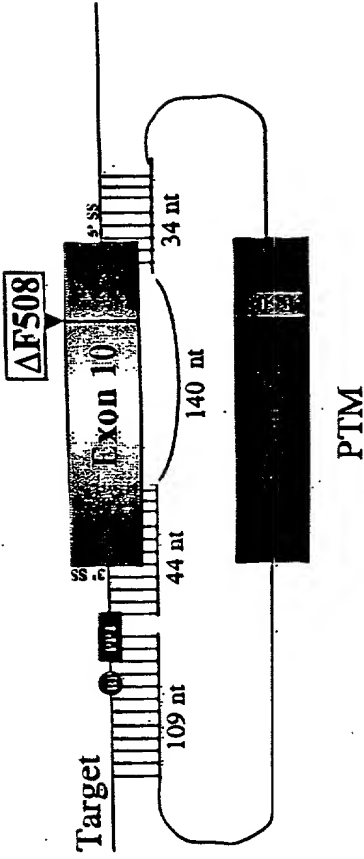


Figure 30

INTRON

**PTM with a long binding domain masking  
two splice sites and part of exon 10  
in a mini-gene target.**



ACGAGCTTGCTCATGATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCCG  
GCCGCATCAGCTTTTCAGCCAAATTACGTTGGATCATGCCGGTACCATCAAGGAGAACATTAAT  
CTTCGGCGTCAGTTACGACGAGTACCGCTATTCGCTCGGTGATTAAGGCCCTGTCAGTTGGAGGAG

### MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (**bold and underlined**).

Figure 31

IRON

Sequence of a double  
*trans*-spliced product

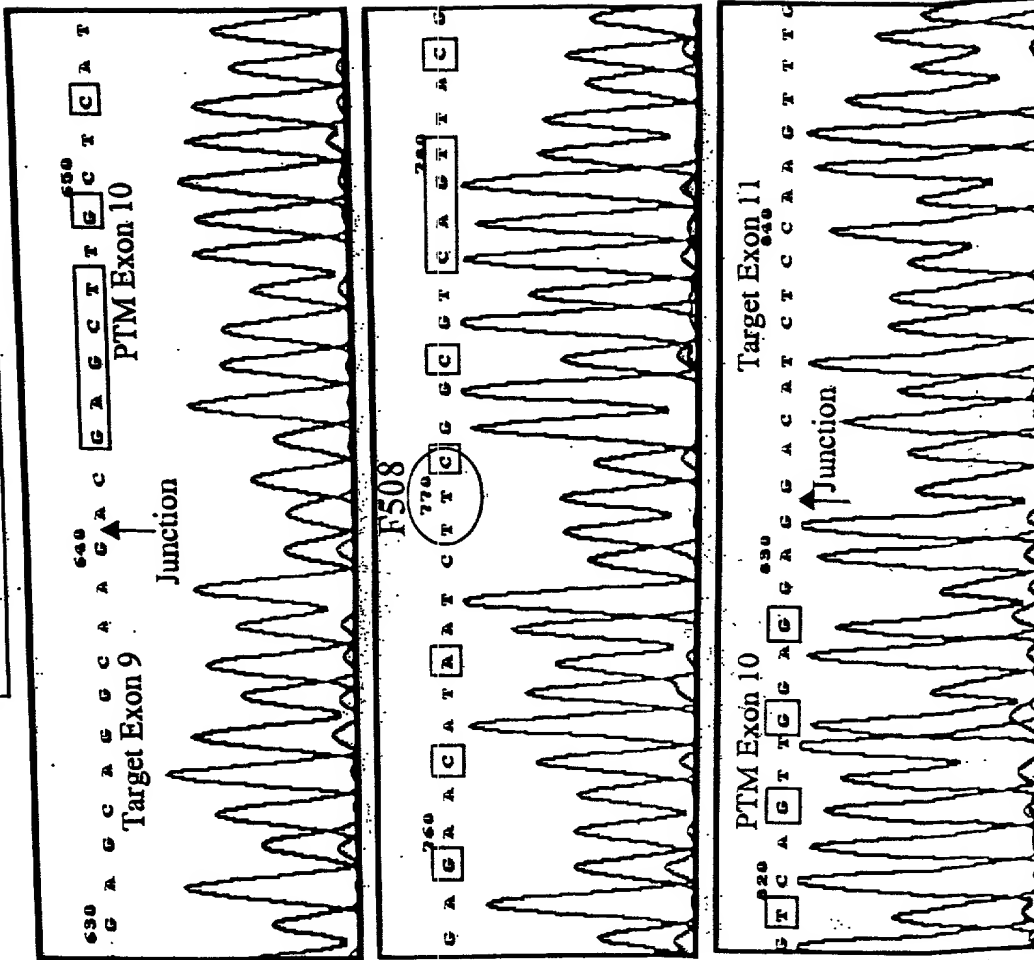


Figure 32

# CFTR Repair: 5' Exon Replacement

Schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target.

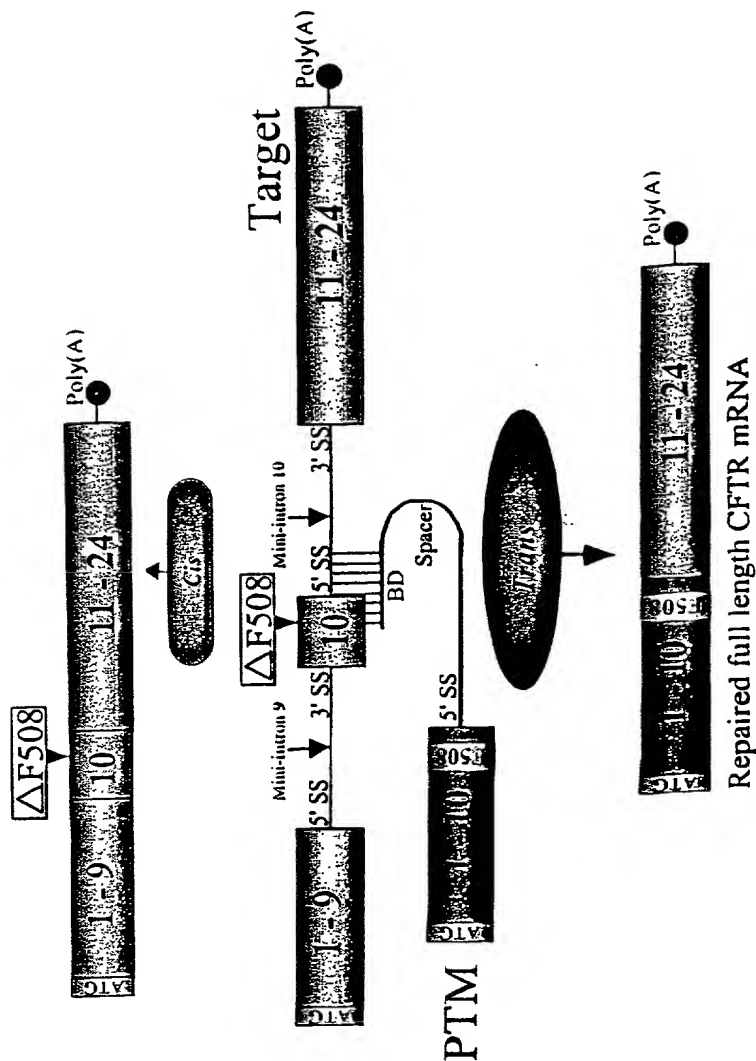
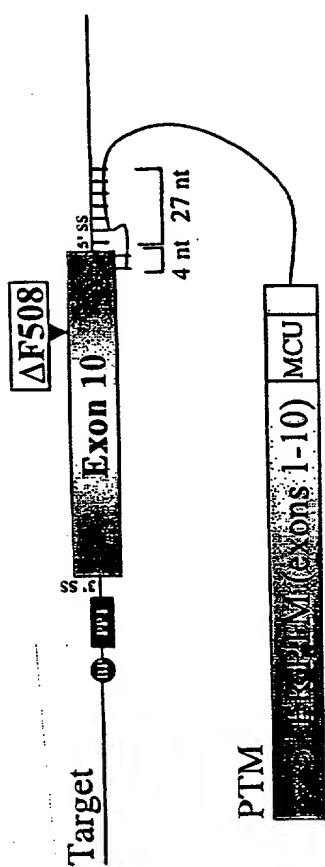
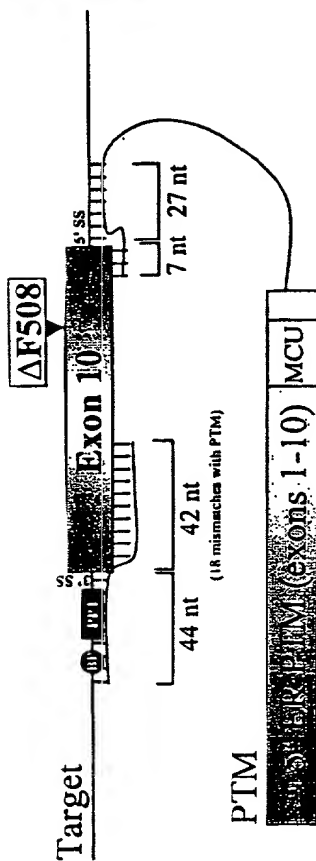


Figure 33

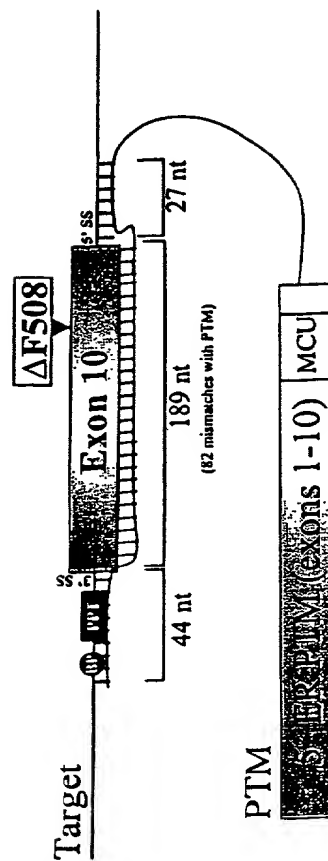
11/11/14



A



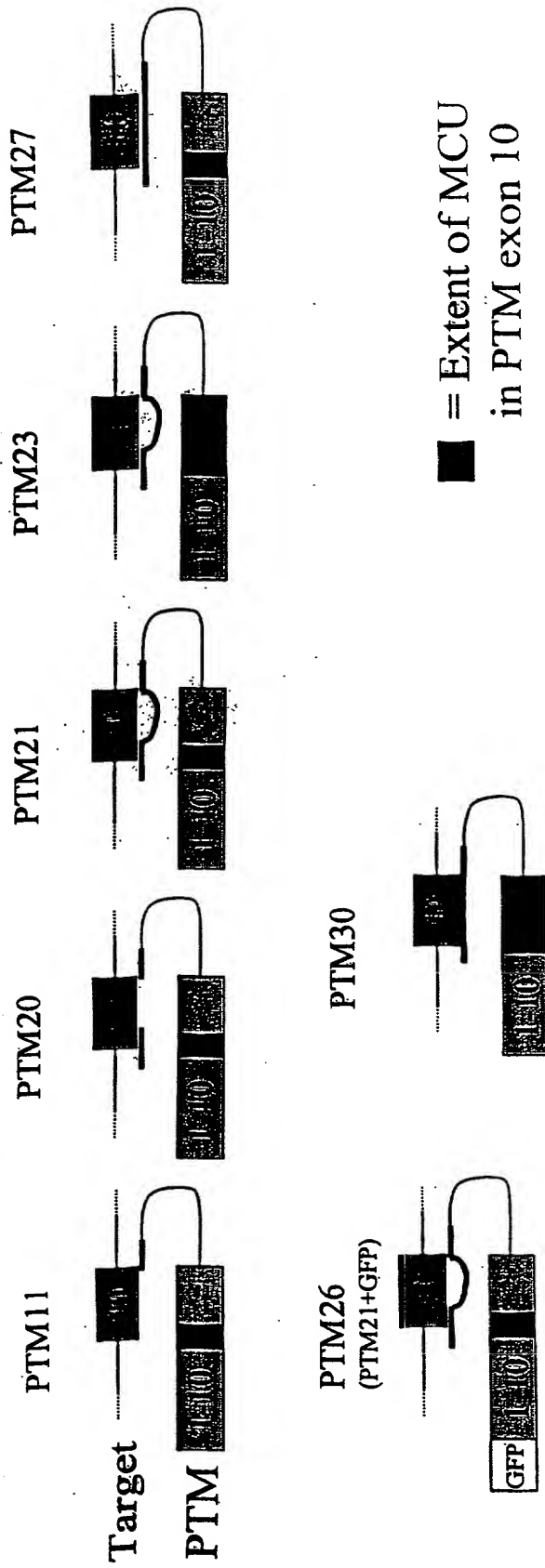
B



C

Figure 34





# MCU in exon 10 of PTM

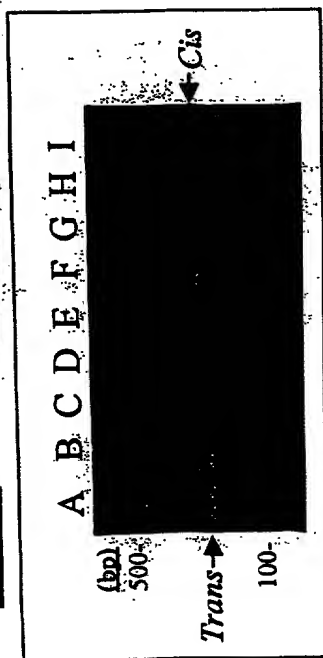
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGTCTCATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAA CATTCCG  
GCCGCATCAGCTTTTGCAGCCAA TT CAGTTGGATCATGCCCGGTACCATCAAGGAGAACATAAT  
CTTCGGCGT CAGTTACGACGAGTACCGCTATCGCTCGGTGATTAGGCCCTGT CAGTTGGAGGAG

Figure 35

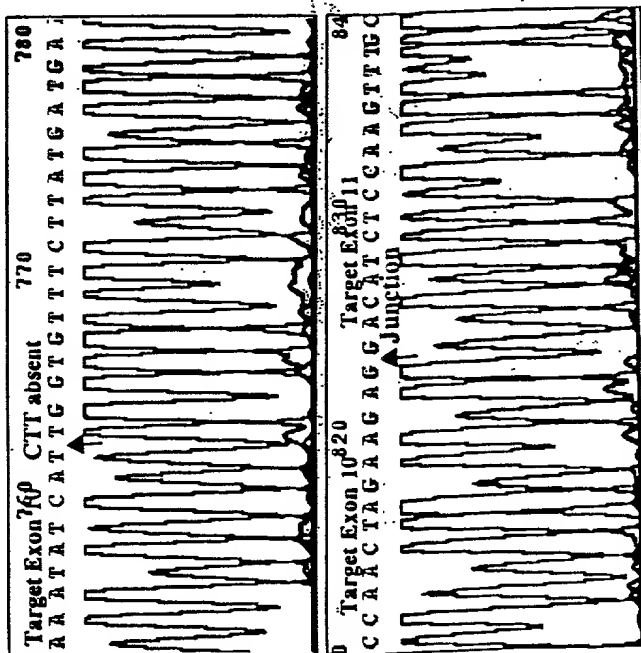
PTM

Target

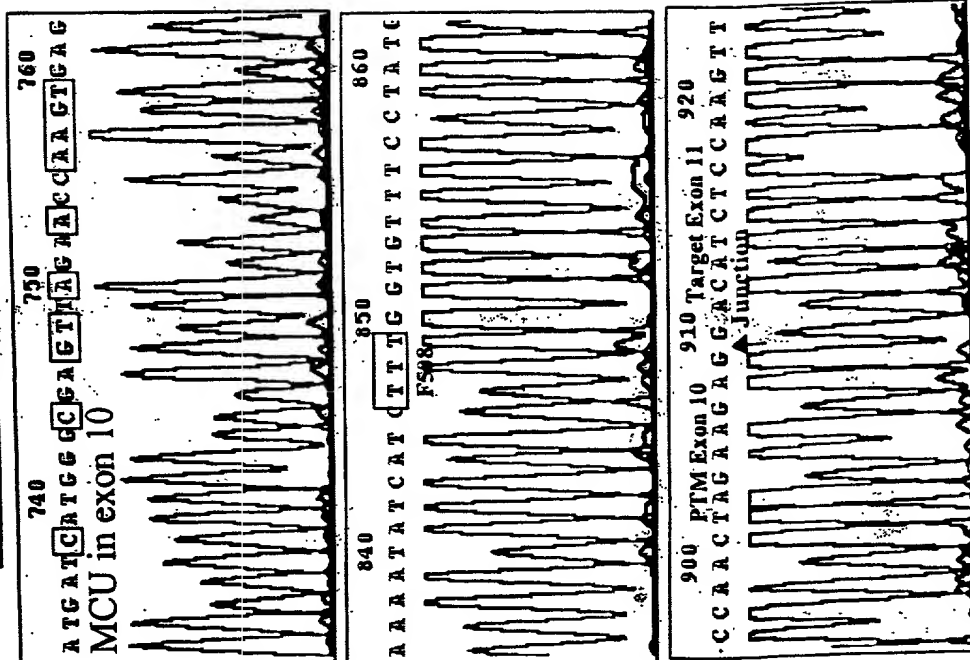


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# **A.** *Cis-spliced product* [Primers CF1 + CF111]



# **B.** *Trans-spliced product* [Primers CF93 + CF111]



A

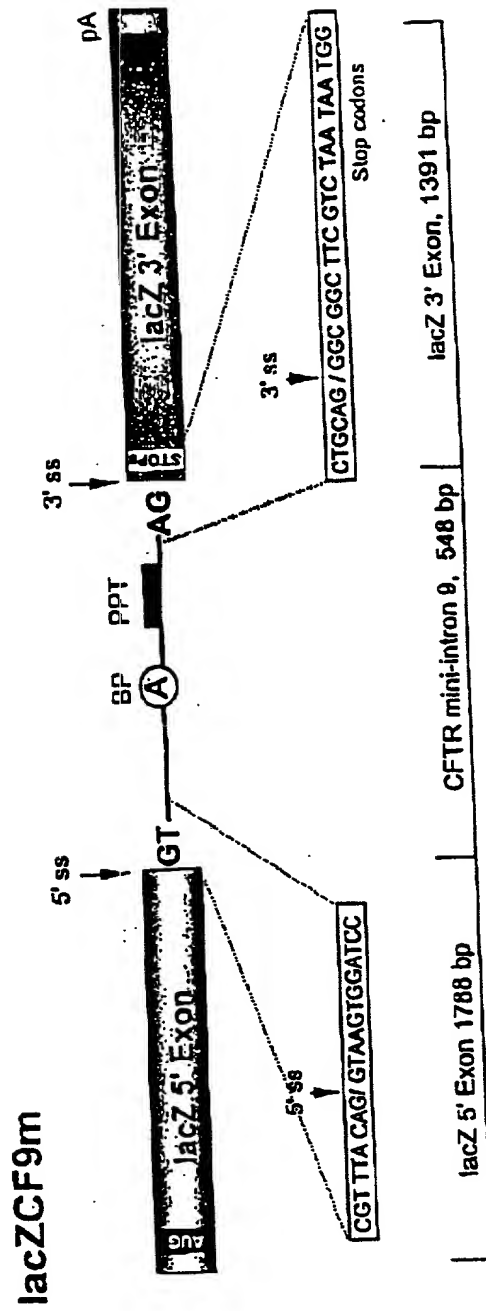


Figure 37 A

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bd 7 87



C

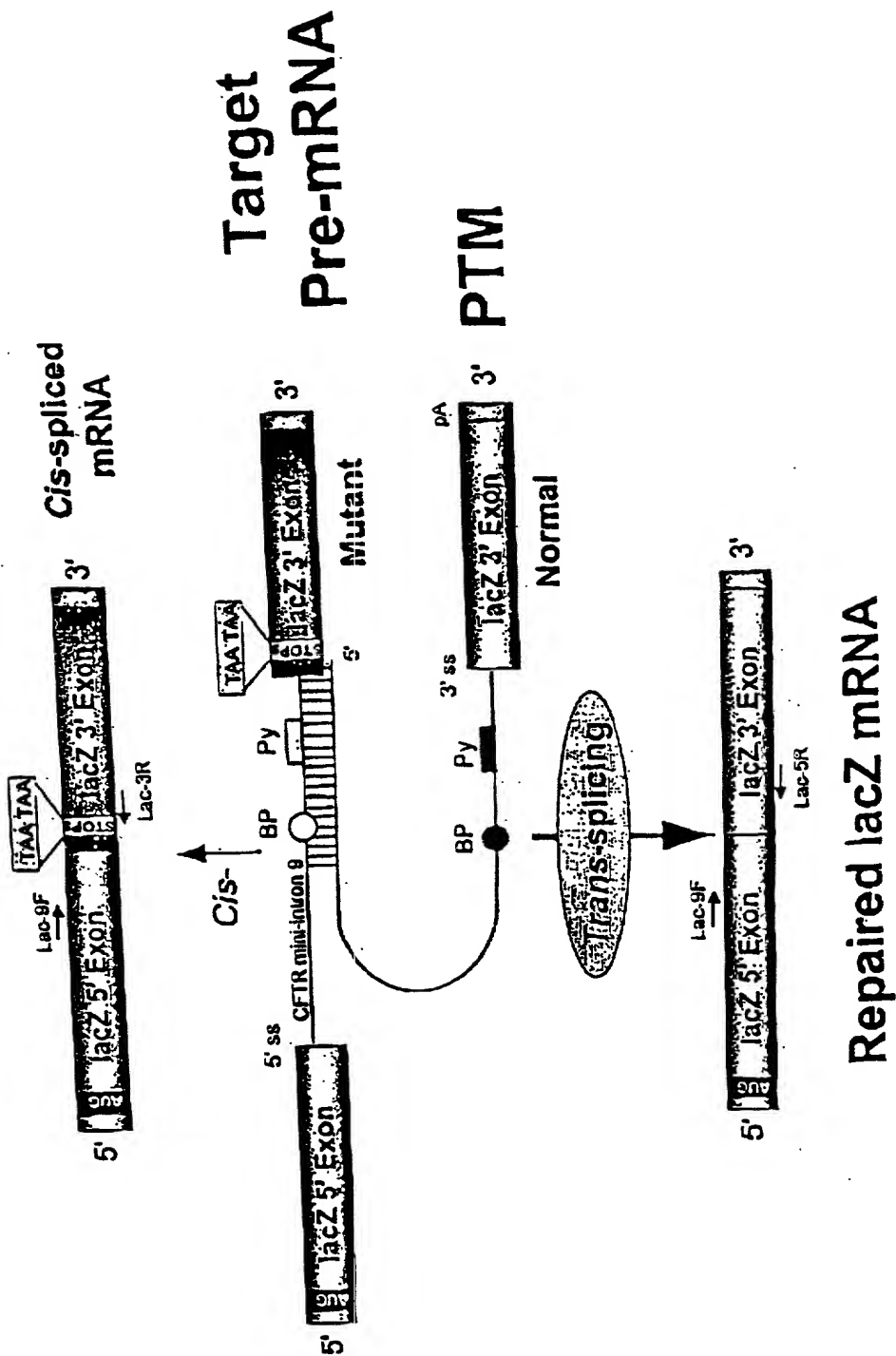


Figure 37C

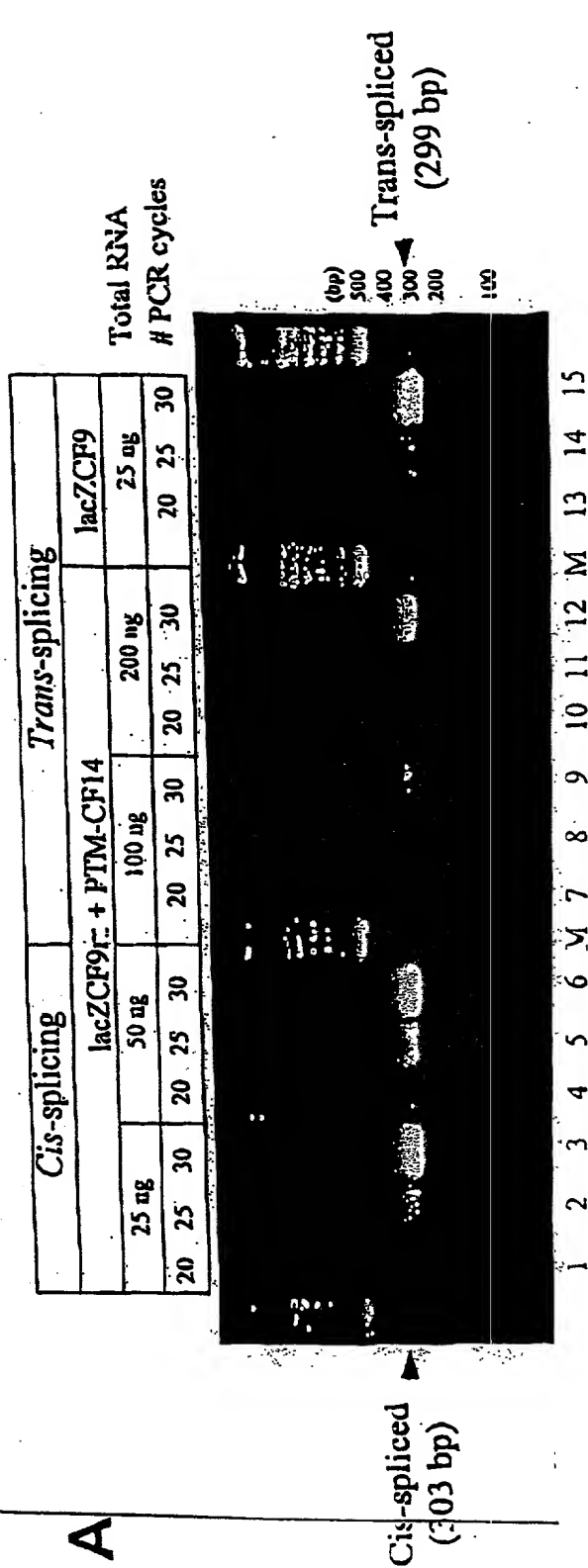
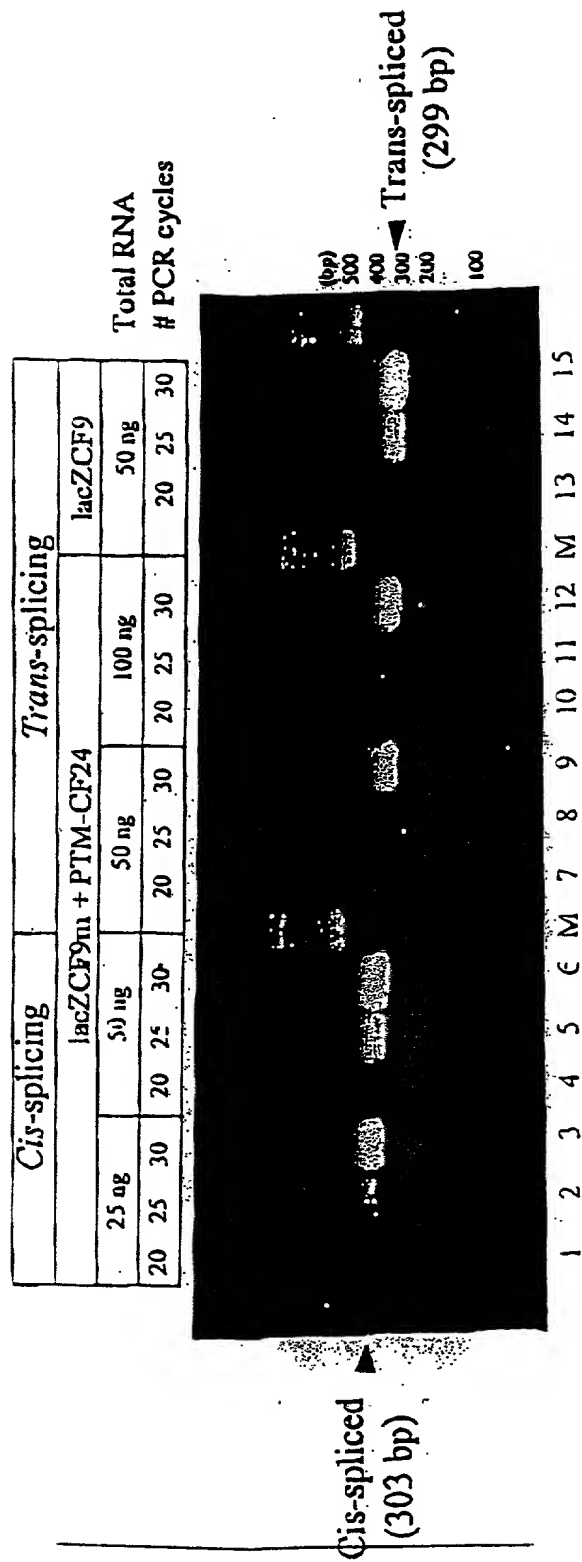


Figure 38A



B

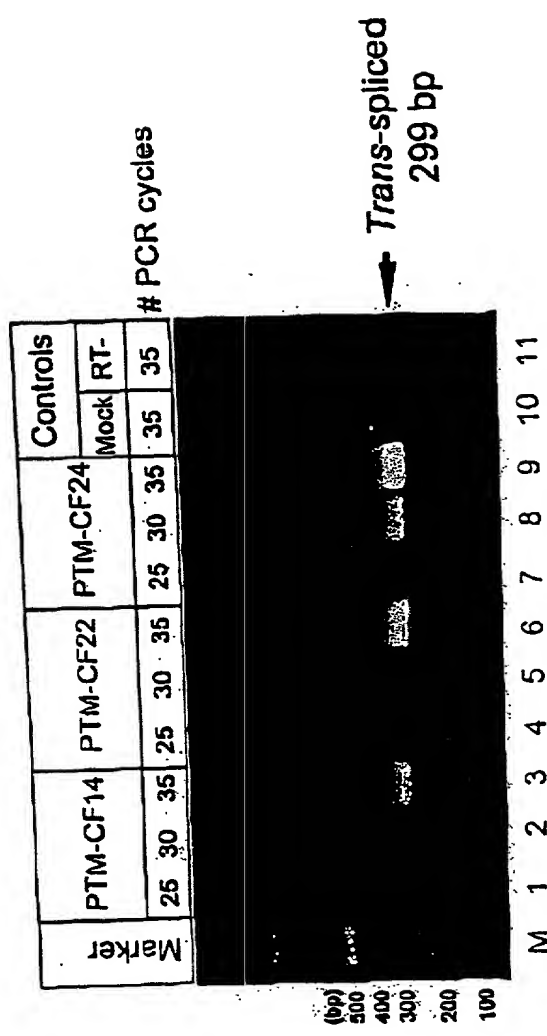


Figure 38B

106280" 2644660

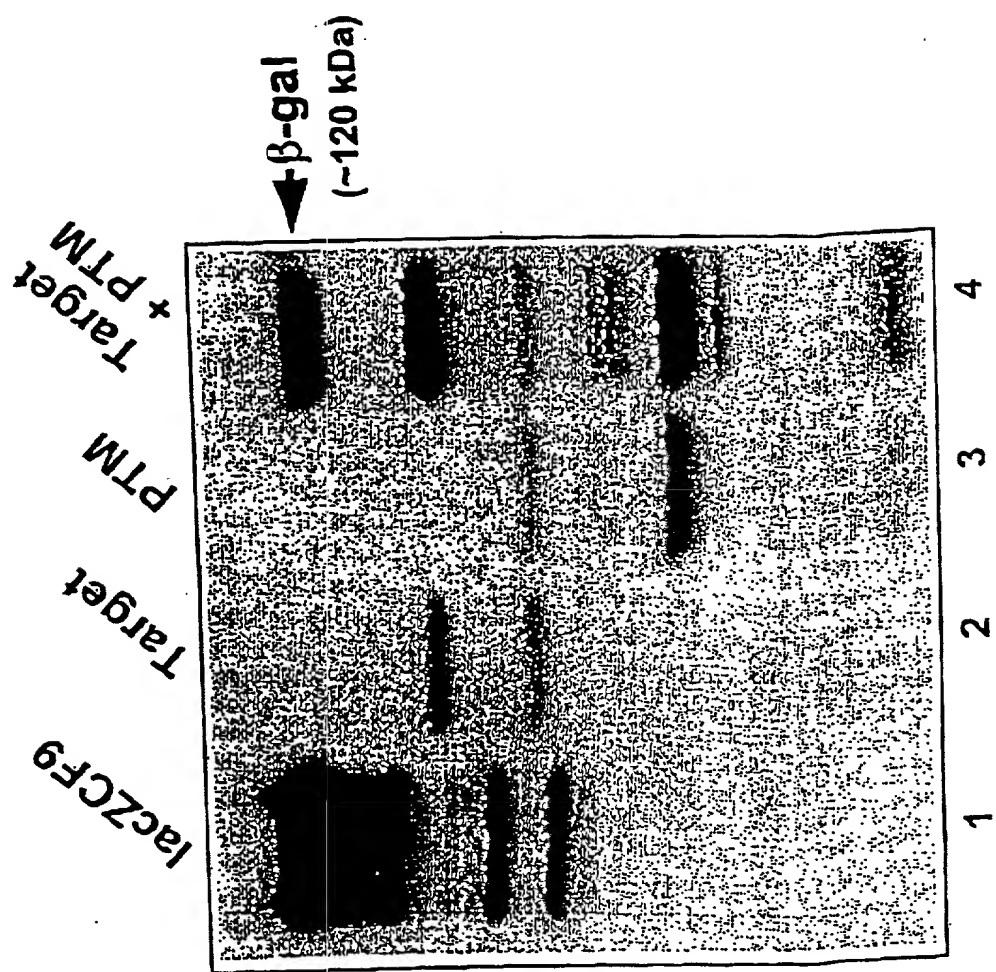


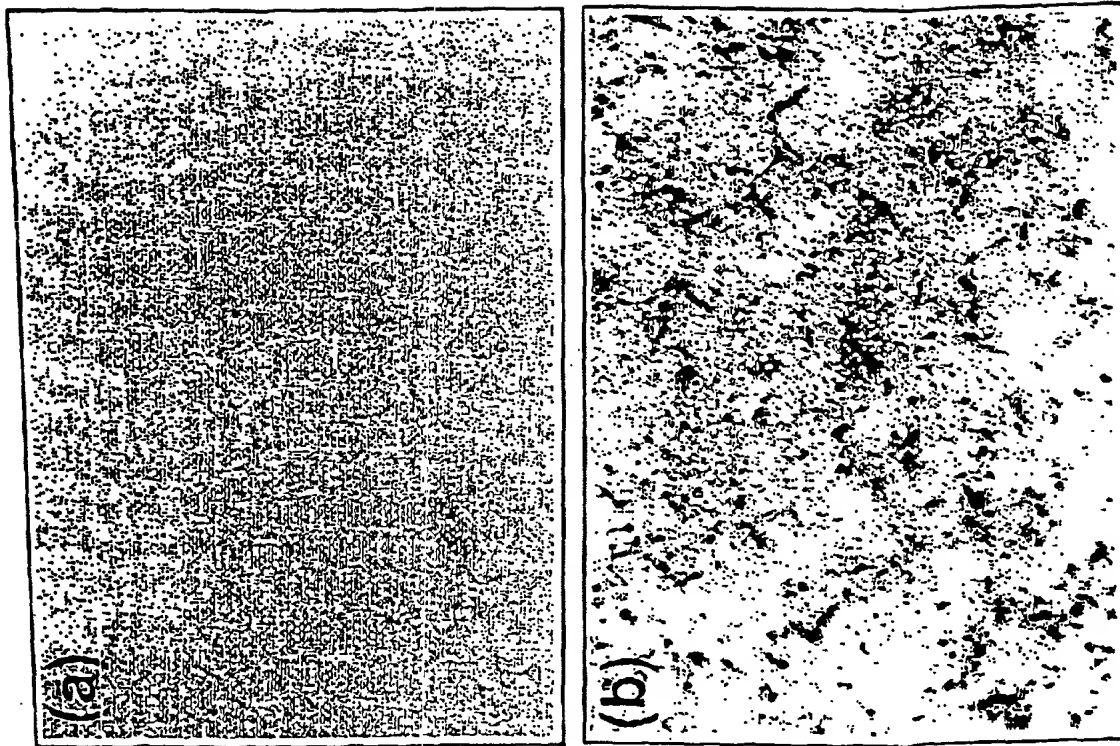
Figure 39

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Figure 40A



TOP SECRET 25414660

B

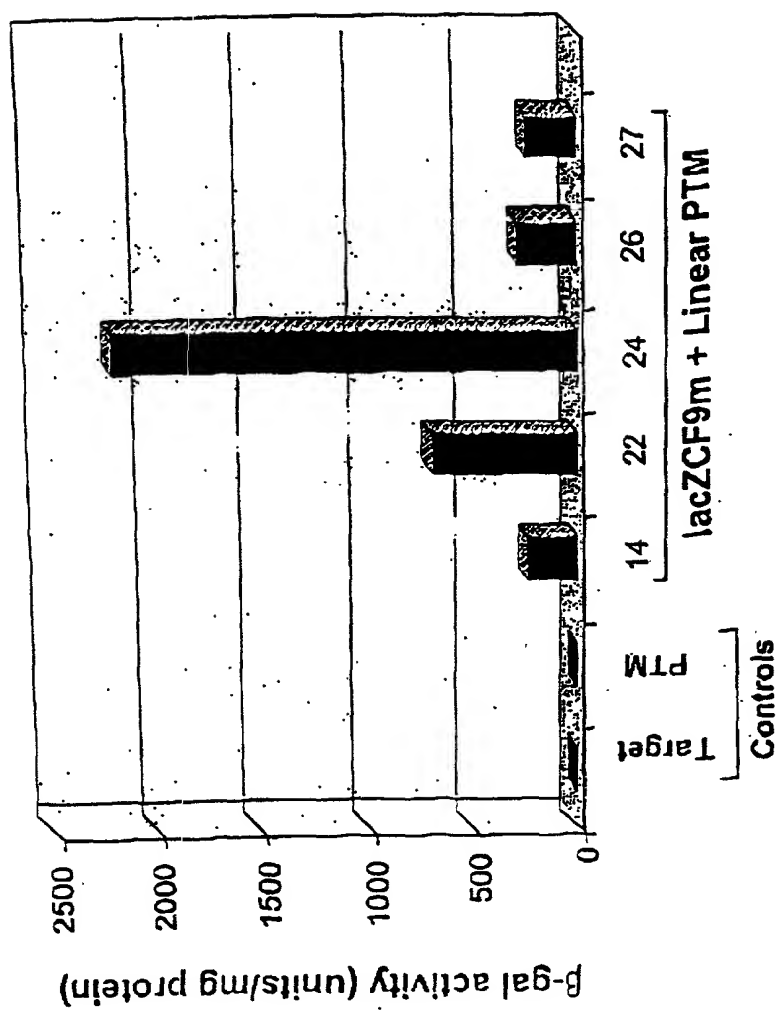


Figure 40B

C

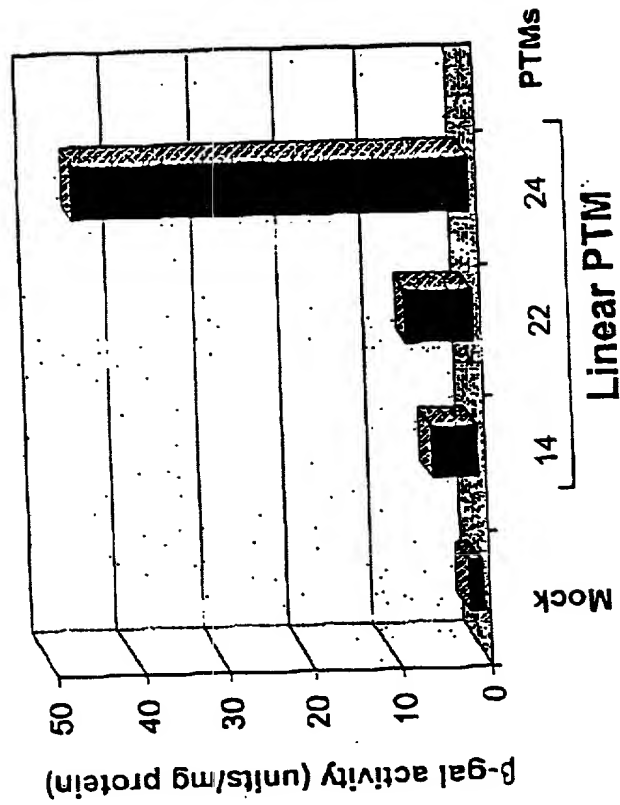


Figure 40C

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A

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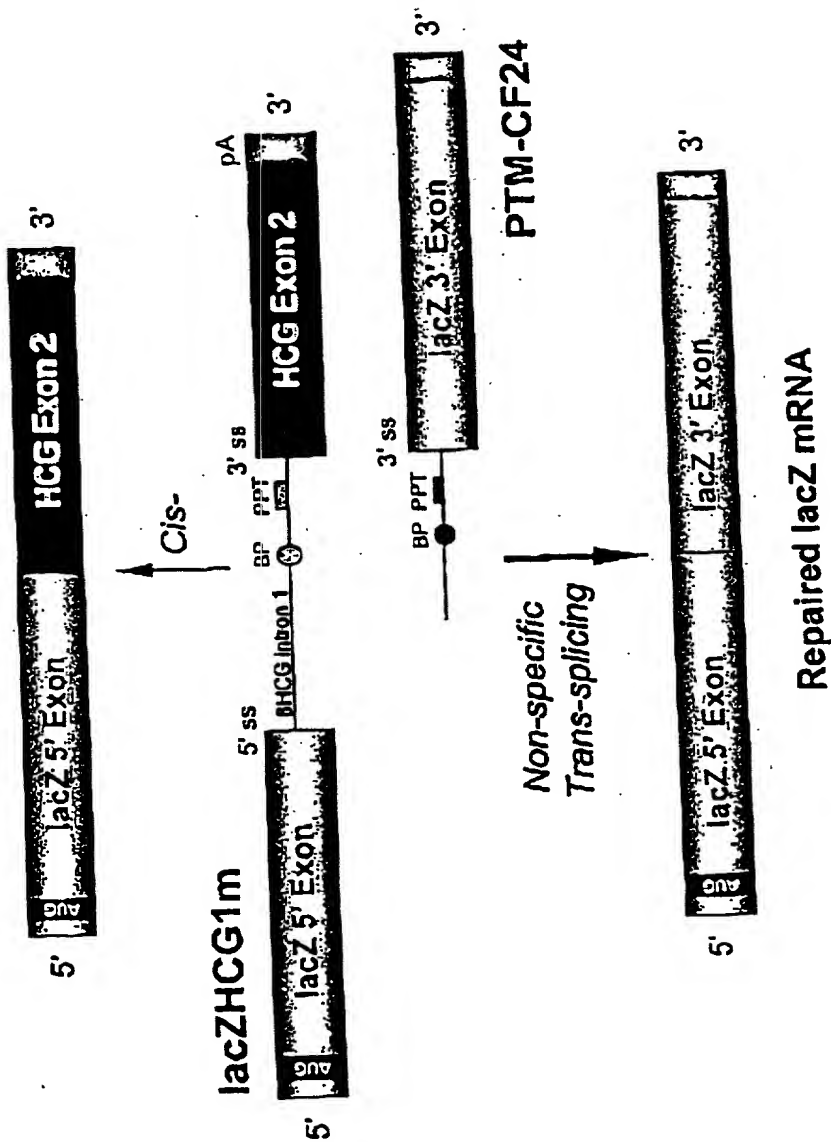


Figure 41A

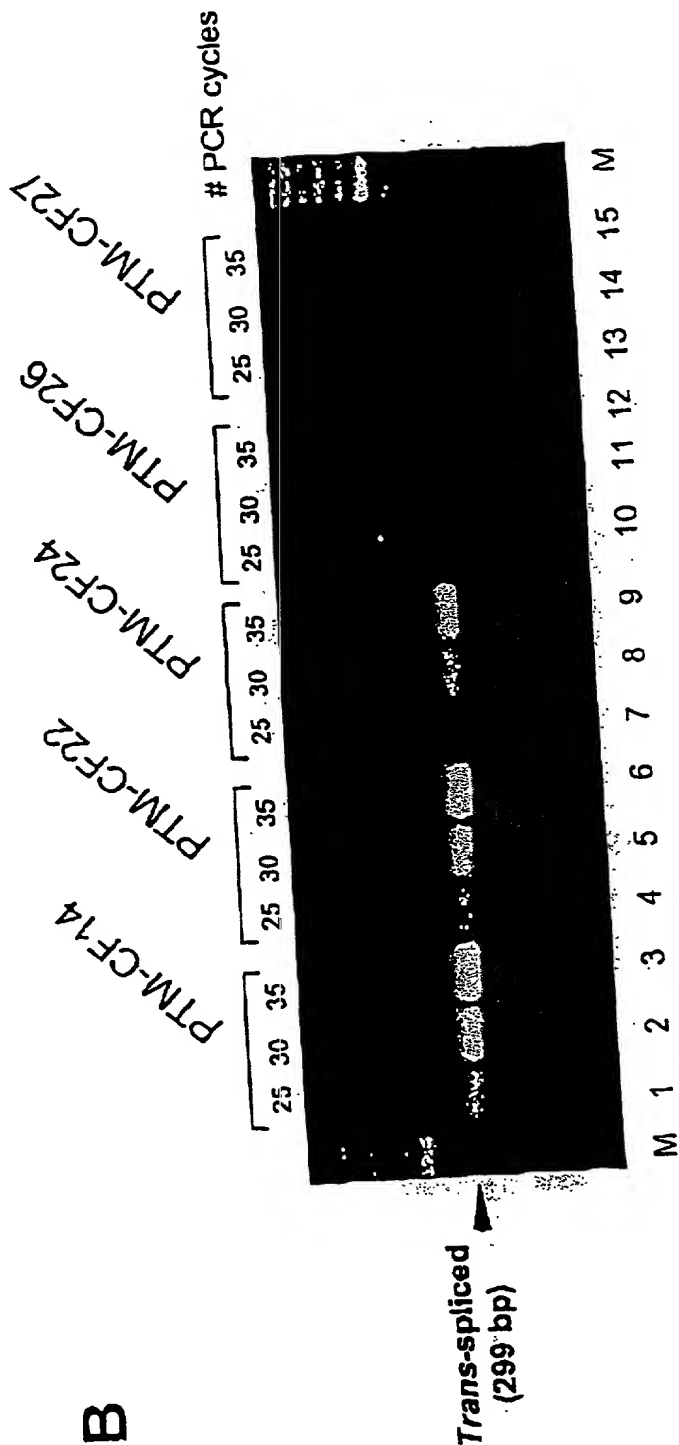


Figure 4B

C

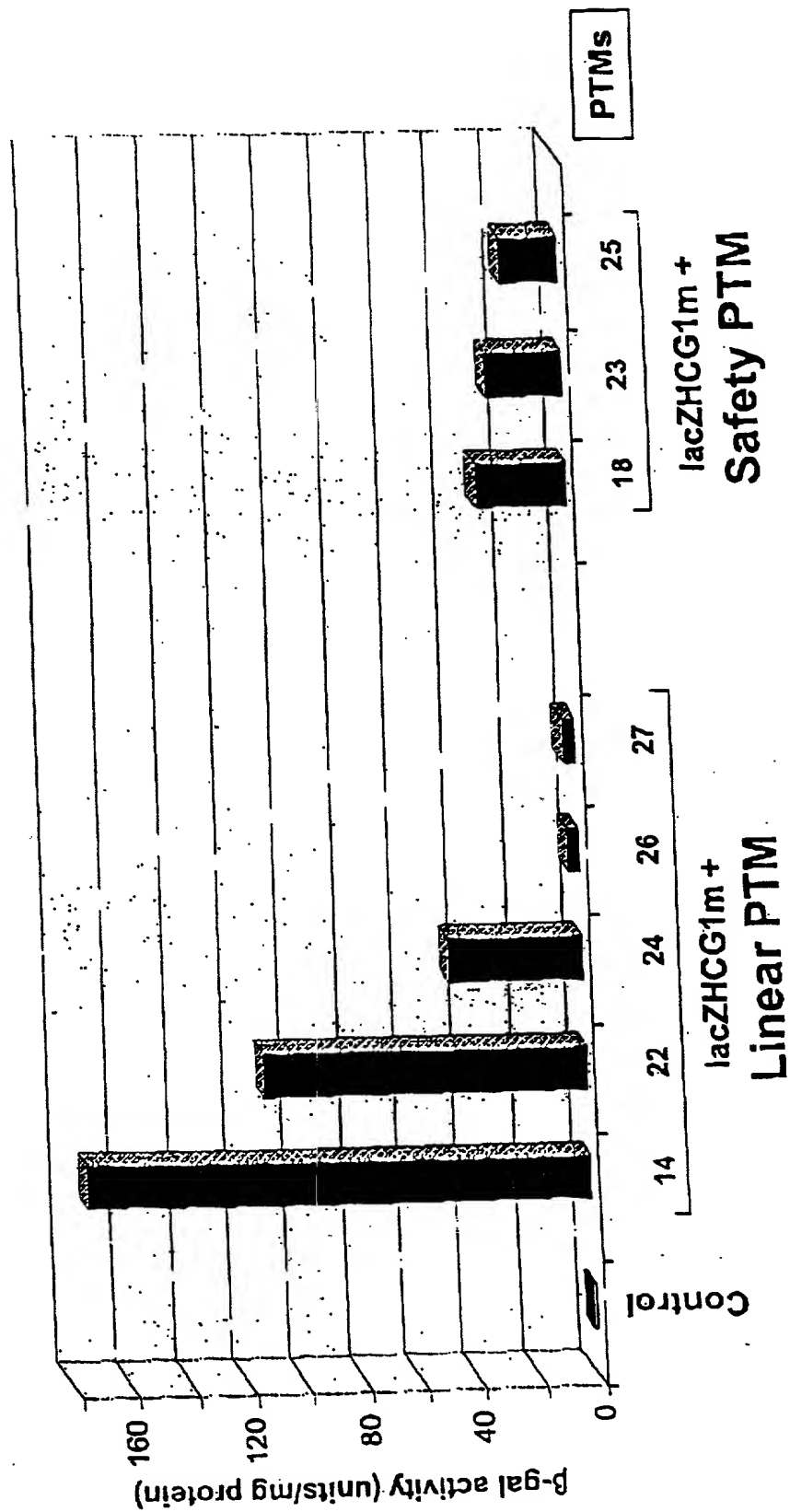


Figure 4C

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Exons 1-10

ATGCAGAGGTGCGCTCTGGAAAAGGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG  
 GATACAGACAGCGCCTGGAATTGTGAGACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT  
 GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAACTCATTAATGCCCTTCGGCGATGTTTTTCTGG  
 AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA  
 TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTATCTAGGCATAGGCTTATGCCTTCTCTTTAT  
 TGTGAGGACACTGCTCCTACACCCAGCCATTTTTGGCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT  
 TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTTGTAGTCTCCTTT  
 CCAACAACCTGAACAAATTTGATGAAGGACTTGCAATGGCACATTTCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT  
 CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCTGTAGTCTCTTGCCCTTTTTTCAG  
 GCTGGGCTAGGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG  
 AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA  
 AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTCTTCTCAGGGTTCTTT  
 GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGGAAATATTACCACCATCTCATTCT  
 GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTGGAGCAATAAA  
 CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG  
 AATGTAACAGCCTTCTGGGAGGAGGGATTGGGGAATTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAAACTT  
 CTAATGGTGATGACAGCCTCTTCTCAGTAATTTCTCACTTCTTGGTACTCCTGTCTGAAAGATATTAATTTCAAGAT  
 AGAAAGAGGACAGTTGTTGGCGGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGGCGAG  
 TTAGAACCAAGTGAAGGCAAGATCAAACTTCCGGCCGCATCAGCTTTTGAGCCCAATTGAGTTGGATCATGCCCGGTA  
 CCATCAAGGAGAACTAATCTTCGGCGTCAAGTACGACGAGTACCGCTATCGCTCGGTGATTAAAGCCTGTGAGTTGGA  
 GGAG


Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTGGGCTTCGATACGCTAAGATCCACCGG  
TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG  
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATCTTCCACTGT  
GCTTAATTTTACCCTCTGAATTTCTCATTCTCCATAATCATCATTACAACCTGAACTCTGGAAATAAAACCCATCATT  
ATTAACCTCATTATCAAACTCAGCT

SCANNED, #

Figure 42

153 bp PTM24 Binding Domain:

Nhe I                      153 bp BD underlined  
GCTAGC-GACGAAGCGCCCTCAGCTCAGGATTCACCTTGCCCTCCAATTATCATCCTAAGCAGAAAGTGTATA  
TTCTTATTGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAATACTTCCTGTTTCACCTACTCTGCTATGC

Sac II  
AC-CCGCGG

68 7 09

Figure 43 A



Figure 43B

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106280" 264 F4660

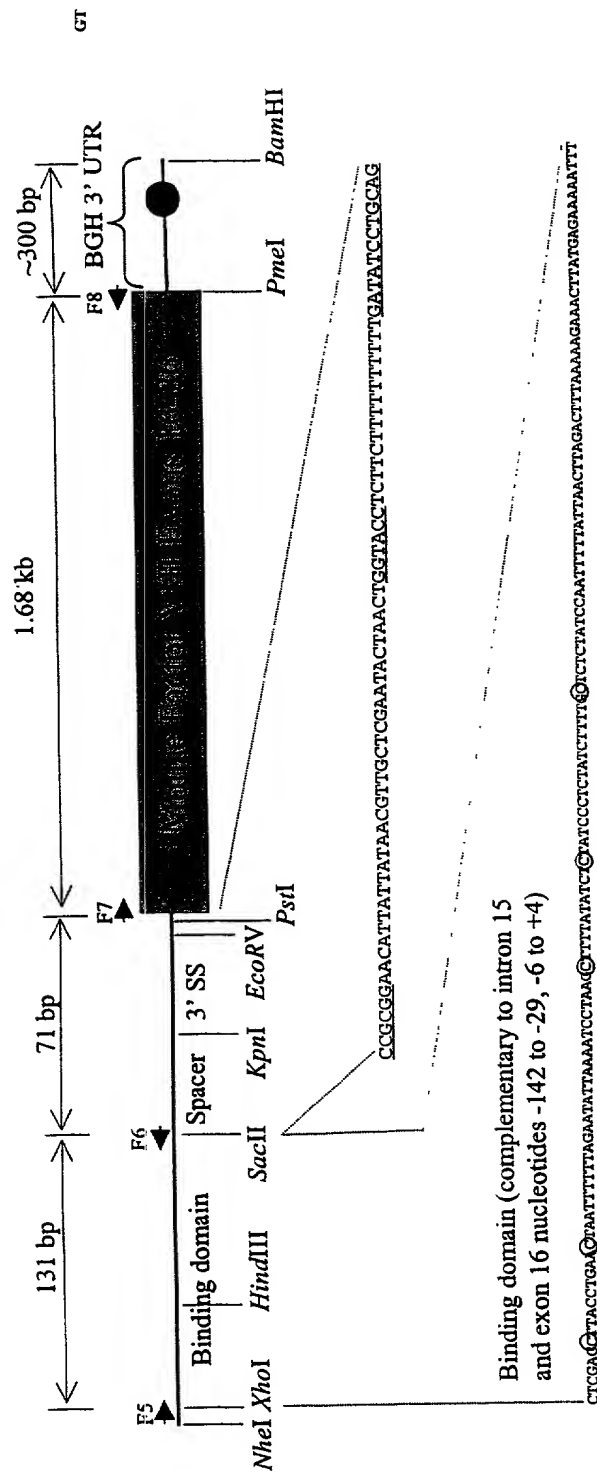


Figure 44 A

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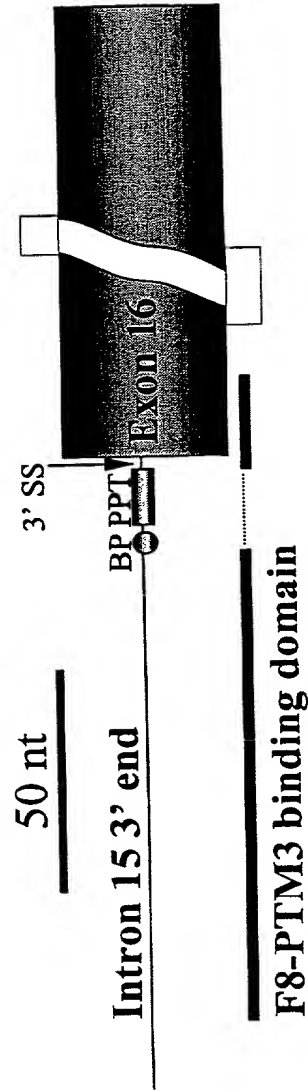
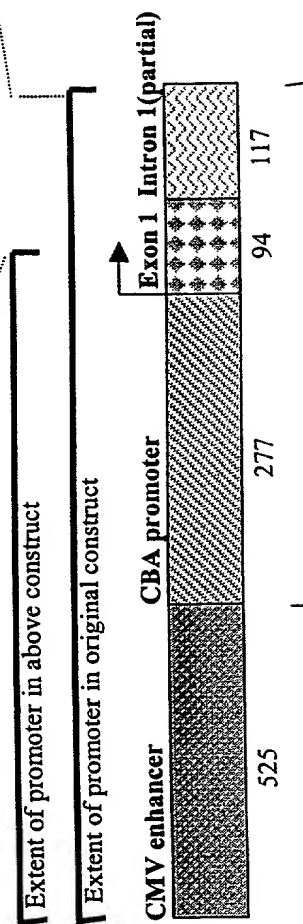


Figure 44 B

Nucleotide changes are shown in blue  
 Boxed = CAT box, TATA box  
 Boxed + Arrow = Transcription Start  
 Oval = Downstream elements  
 Bold = Binding domain  
 Italicized = Spacer+PP1+BP+AG dinucleotide

Sequence not included in construct

CGCCGCCCTCGGCGCCGCCGCCGCCGGCTCTGACTGACCGCGTTATC'CCACAGGTGAG  
CGGGCGGGACGGCCCCCTTCTCCTCCGGGCTGTAAATTAGCGCTTGTTTAATGACGGCT

$$\begin{aligned} \text{F13} + \text{F2} &= 235 + 106 = 341 \text{ bp} \\ \text{F13} + \text{F4} &= 235 + 315 = 550 \text{ bp} \end{aligned}$$


Chicken Beta Actin Promoter (including exon 1 and part of intron 1)

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106280" 2644560

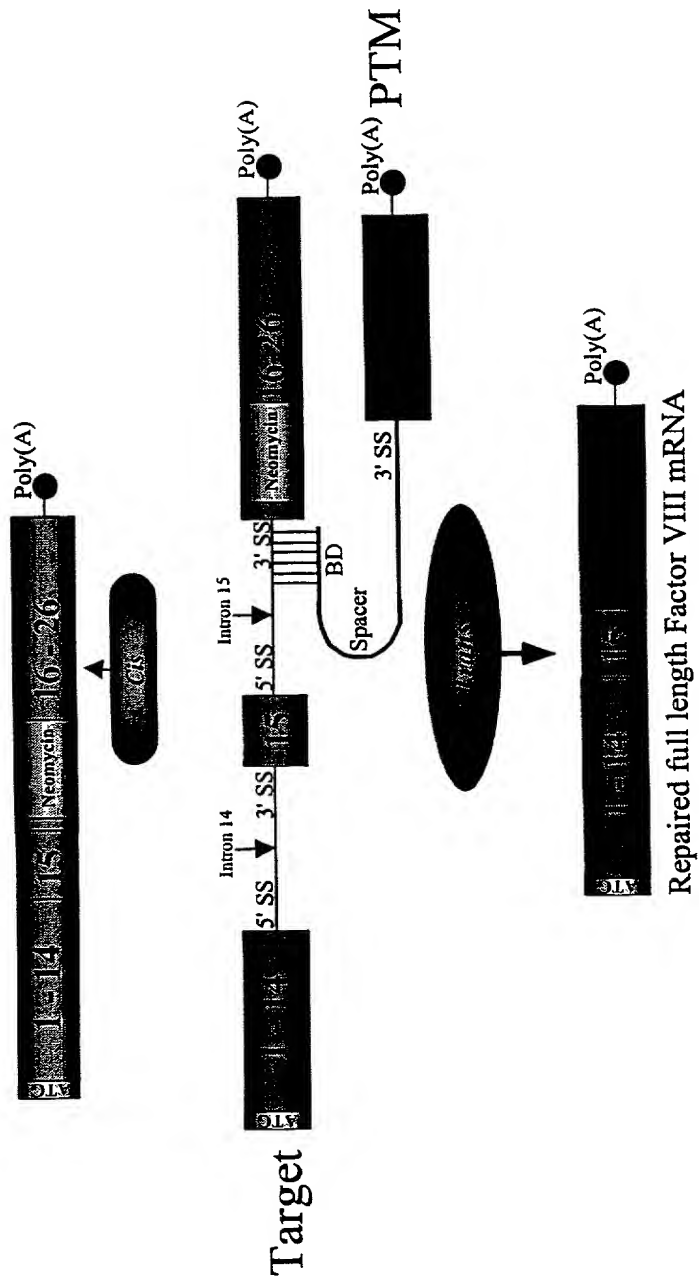
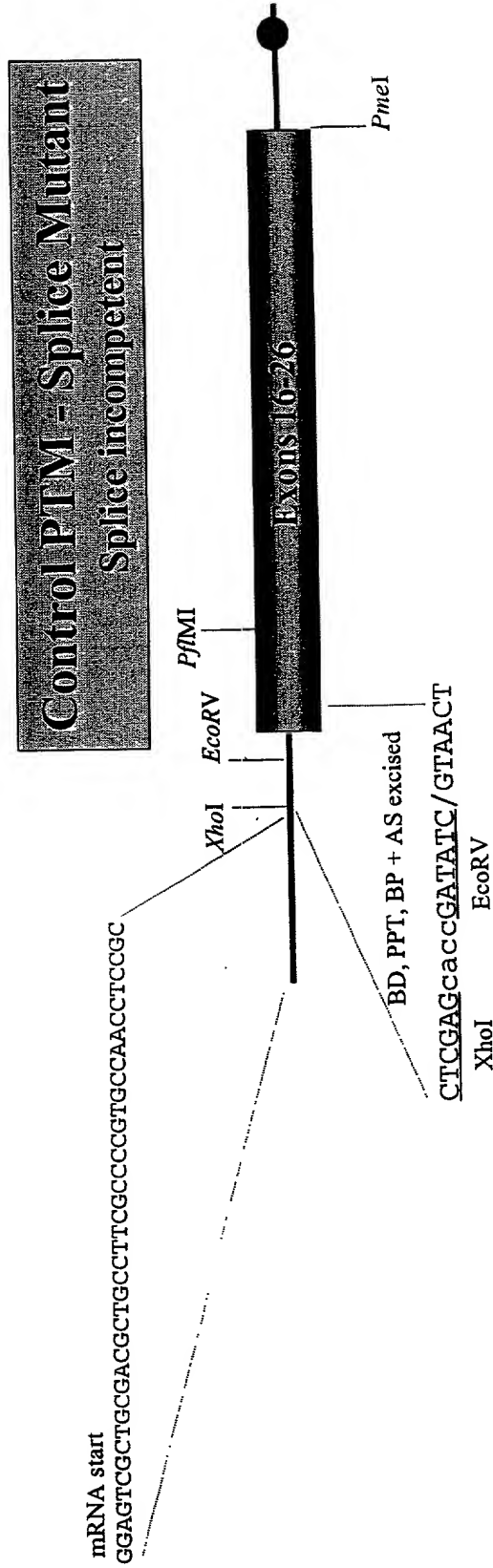


Figure 44D

Figure 45



Method:

Excise TSD and part of exon 16 with

XhoI and PflMI and ligate in a PCR product that:

1) eliminates the TSD and splice acceptor site

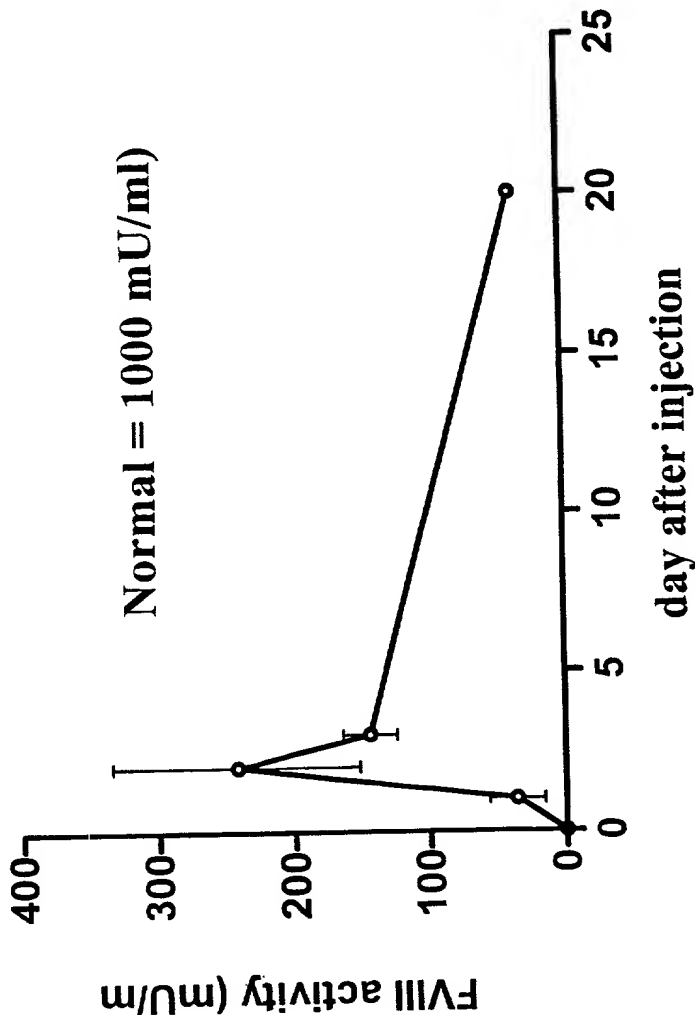
2) inserts EcoRV adjacent to exon 16

3) restores the coding for exon 16

# Repair of Factor VIII

*Preliminary results from one experiment*

FVIII activity in Exon 16 FVIII-KO mice  
after IV PTM-FVIII intraportal infusion  
(100ugDNA)(n=3)



## METHODS

Inject plasmid intraportally

↓

Sample blood (1, 2, 3, 20 d)

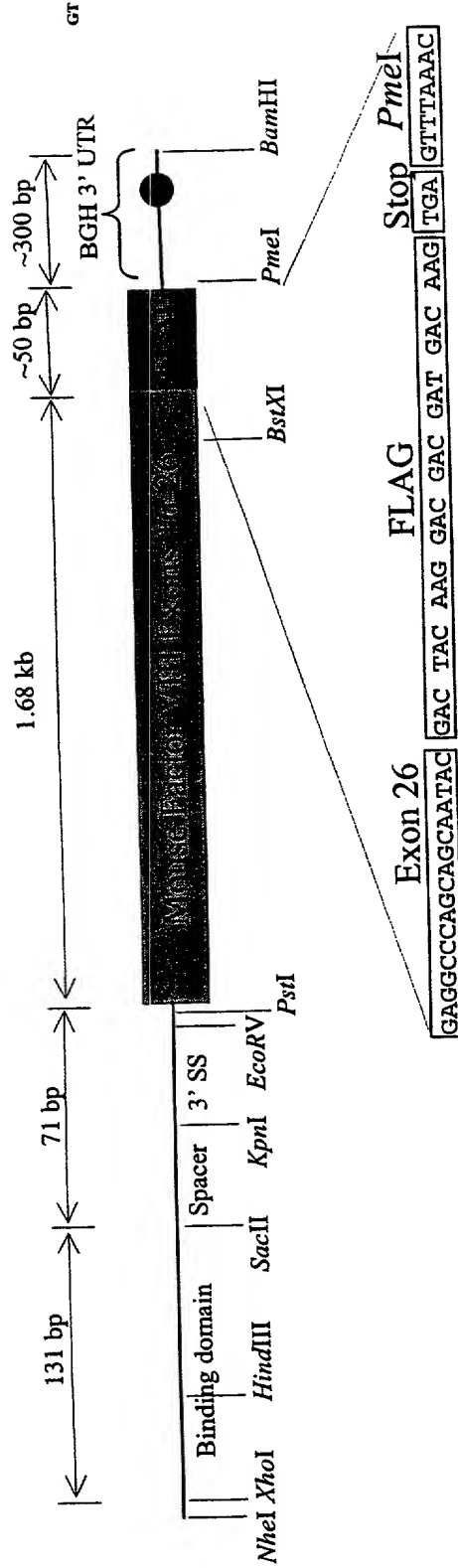
↓

Assay for factor VIII activity

Figure 46

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Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH = bovine growth hormone 3' UTR; Binding domain = 125 bp.



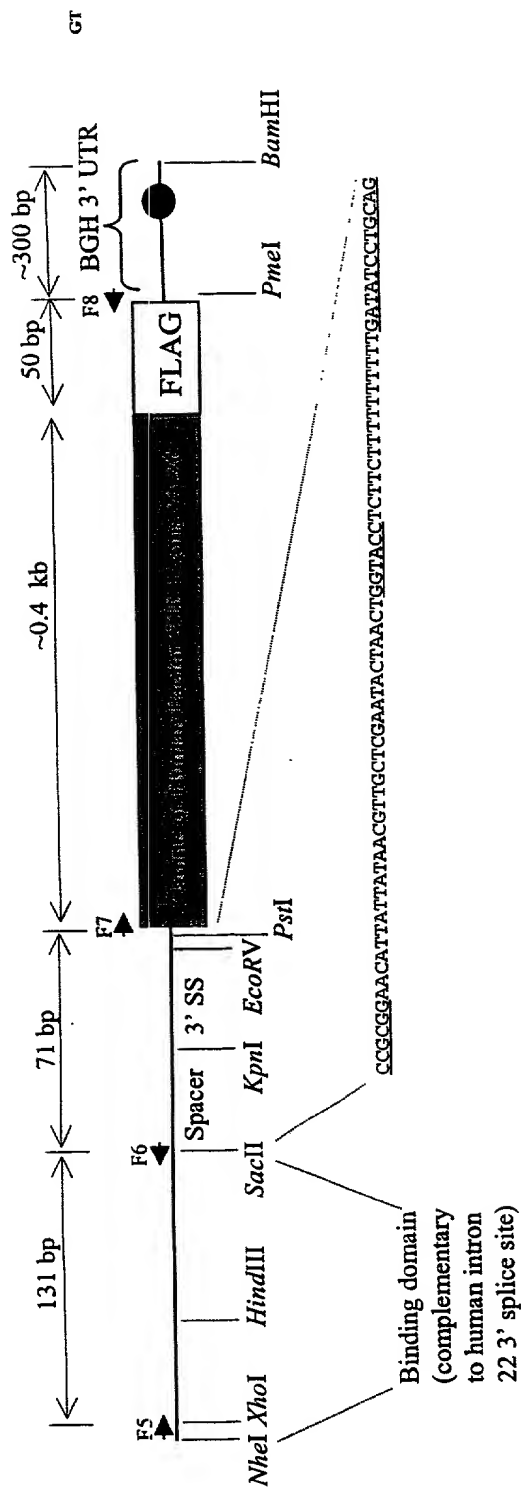
**REFERENCE FOR DESIGN OF FLAG TAG**

Brann T, Kayda D, Lyons RM, Shirley P, Roy S, Kaleko M, Smith T.  
Adenoviral vector-mediated expression of physiologic levels of human factor VIII in nonhuman primates.  
Hum Gene Ther 1999 Dec 10;10(18):2999-3011  
Genetic Therapy, Inc., a Novartis Company, Gaithersburg, MD 20878, USA.  
Epitope-tagged B domain-deleted human factor VIII cDNA (flagged FVIII) was evaluated in nonhuman primates.

Figure 47A

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FLAG = C-terminal tag to be used to detect repaired factor VIII protein.

Figure 47B

# Transcription Map of HPV-16

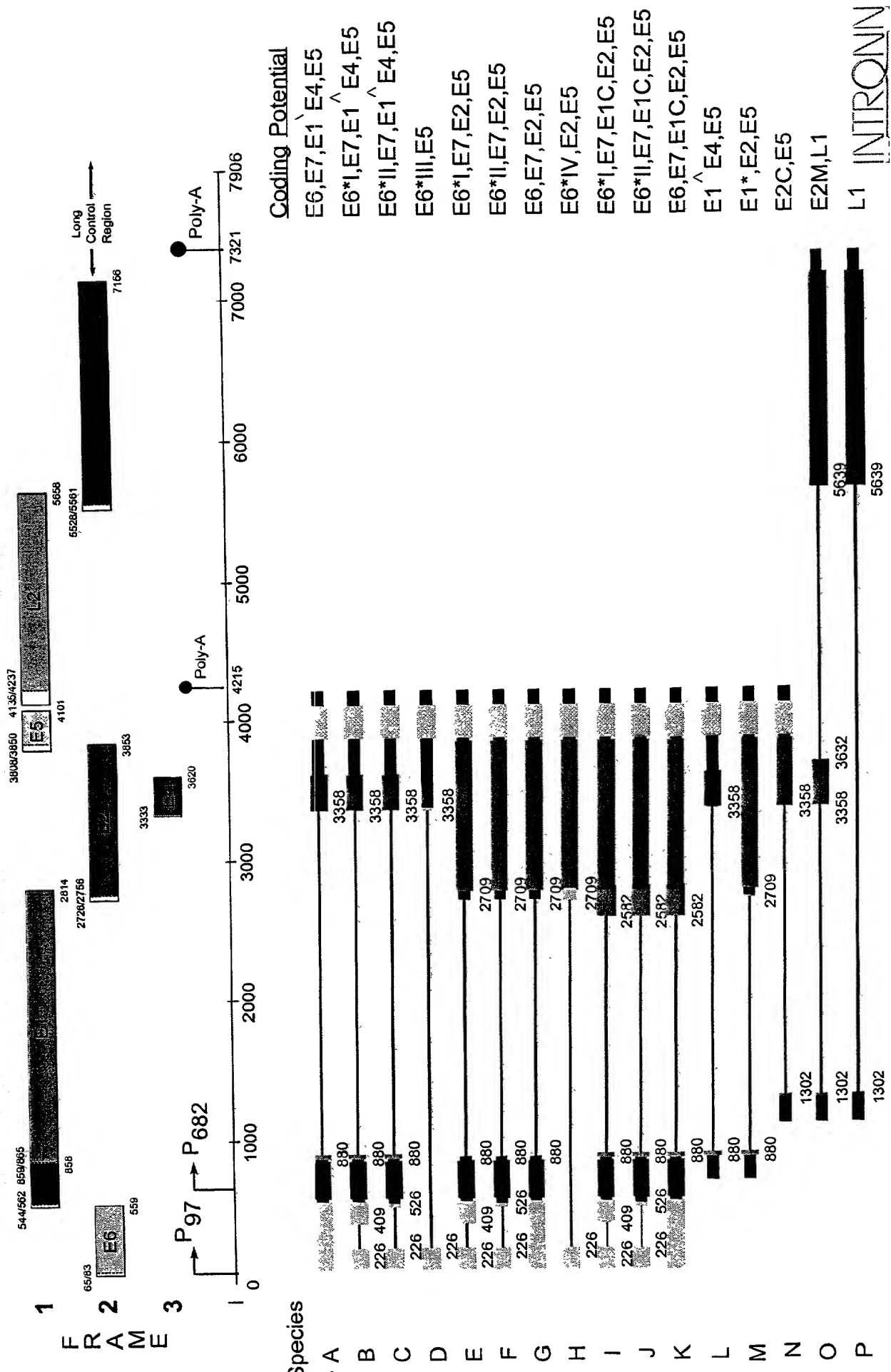
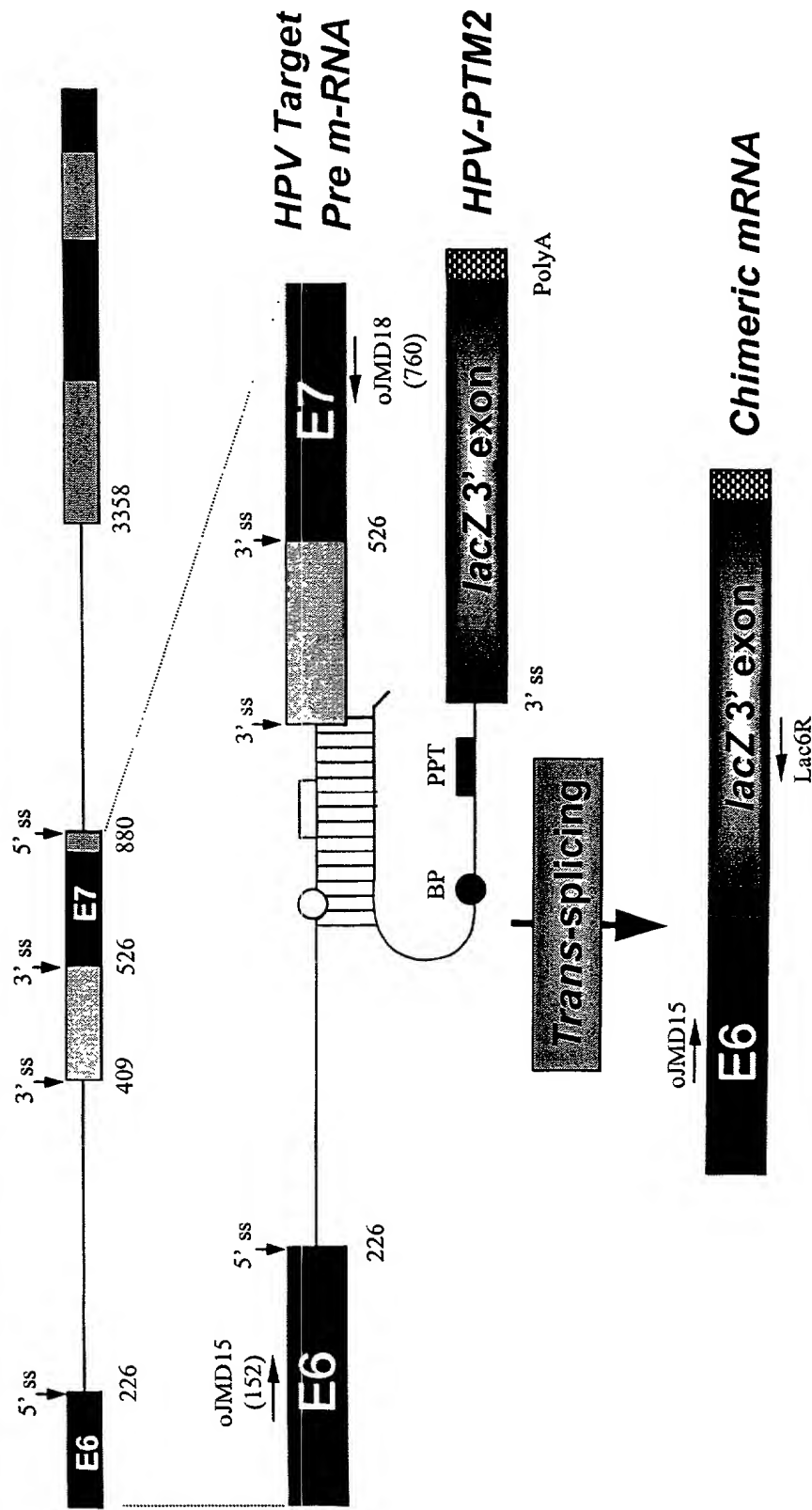


FIGURE 48

# SMART Strategy to Disrupt the Expression of Human Papillomavirus Type 16

## Targeting E6 Exon



SMART Strategy by 3' Exon Replacement: Schematic diagram of HPV-PTM2 binding to the 3' splice site of the HPV type 16 target pre-mRNA

FIGURE 49

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# PTM Design

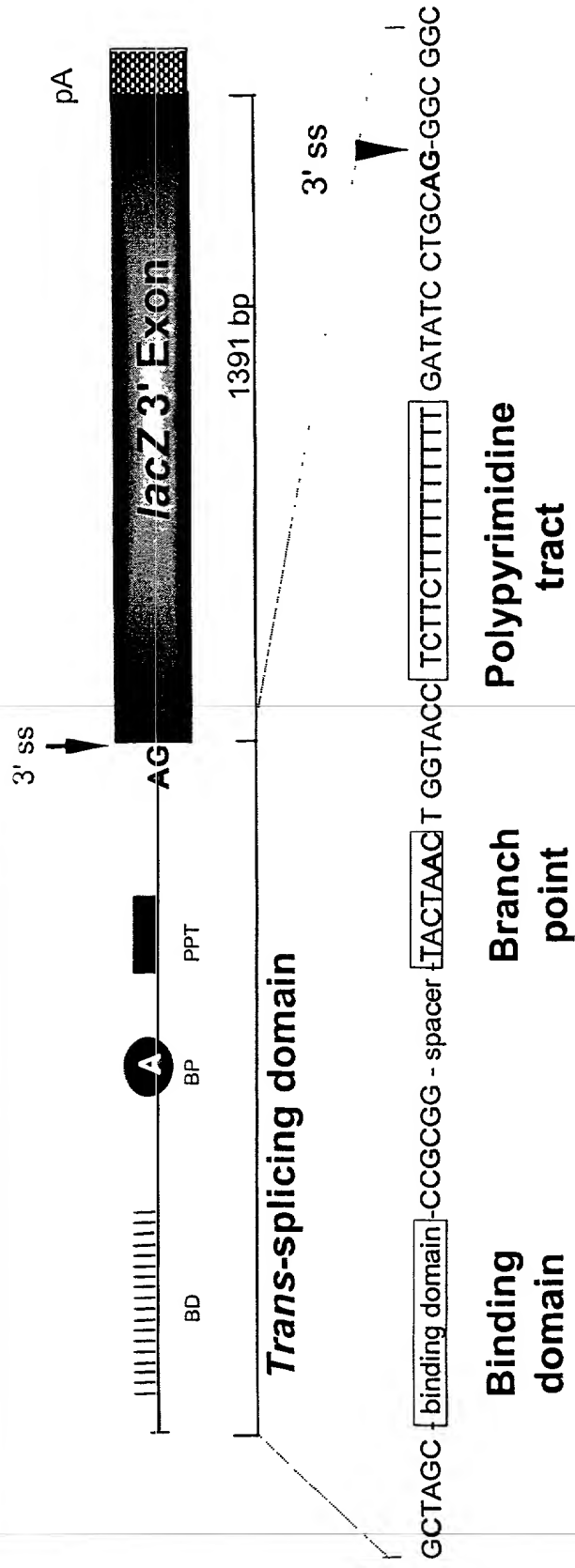
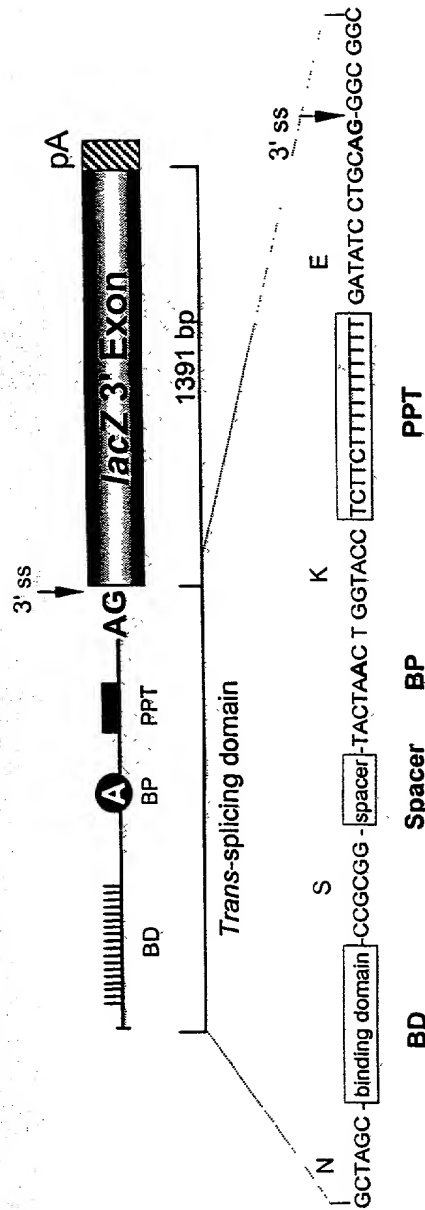


FIGURE 51

INTRONN

HPV-PTM1 with 80 bp binding domain targeted to 3' ss at 409:



Binding domain sequence: CAGTTAATAC ACCTAAATTA CAAATCACAC AACGCTTTGT TGTATTGCTG  
TTCTAATGTT GTTCCATACA CACTATAACA

HPV-PTM2 with 149 bp binding domain targeted to 3' ss at 409:



Binding domain sequence: CAGTTAATAC ACCTAAATTA CAAATCACAC AACGCTTTGT TGTATTGCTG  
TTCTAATGTT GTTCCATACA CACTATAACA ATAATGCTA TACTCACTAA  
TTTTAGAATA AAACTTTAAA CATTATCAC ATACAGCATA TCGATTCCC

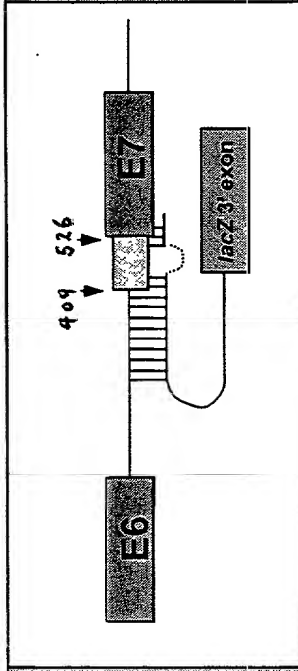
FIGURE 52

INTRONIN

## Binding Domains of HPV-PTM3 and 4

HPV-PTM3 Binding domain (covers both 3' ss at 409 and 526; has 53 bp bubble)

GATGATCTGCAACAAGACATACATCGACCGGTCCA (53 nt bubble) CTTCAGGACACAGTGGCTTTTGAC  
AGTTAATACACCTAAATTAACAATAATCACACAACGGTTTGTGATTTGCAGTTCTAATGTTGTTCCATACACACTA  
TAACAAT



HPV-PTM4 Binding domain (covers both 3' ss at 409 and 526; has 76 bp bubble)

GATGATCTGCAACAAGAC (76 nt bubble) GACACAGTGGCTTTTGACAGTTAATACACCTAATTAACAAATC  
ACACAACGGTTTGTGTATGTCAGTTCTAATGTTGTTCCATACACACTATAACAAT

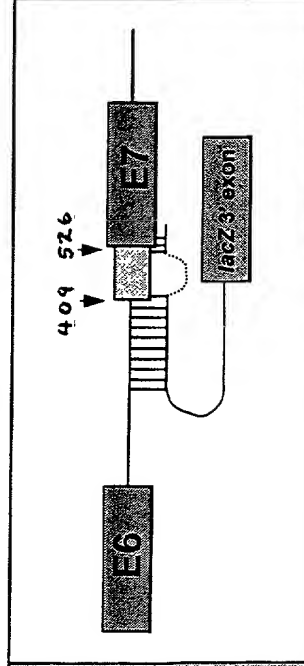


FIGURE 53

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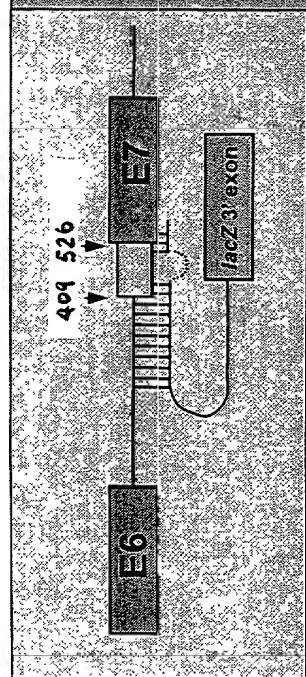
# HPV-PTM5 and 6

**HPV-PTM5**, Binding domain (140 nt, has 53 nt bubble, covers 3'ss at position 409 and 526)

GATGATCTGCAACAAGACATACATCGACCGGTCCA. CTTCAGGACACAGTGGCTTTTGACAGTTAATACACCTAATTAAACAATCACACAACGGT  
TTGTTGTATTGCAGTTCTAATGTTGTTCCATACACACTATAACA

CCGT

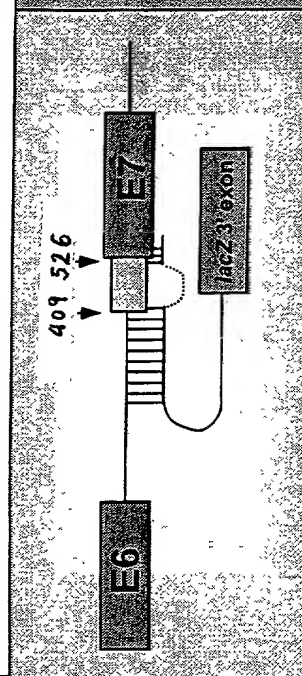
CCGT



**HPV-PTM6**, Binding domain (117 nt, has 76 nt bubble, covers 3'ss at position 409 and 526)

GATGATCTGCAACAAGAC.GACACAGTGGCTTTTGACAGTTAATACACCTAATTAAACAATCACACAACGGTGGTTGTATTGCAGTTCT  
AATGTTGTTCCATACACACTATAACA

CCGT



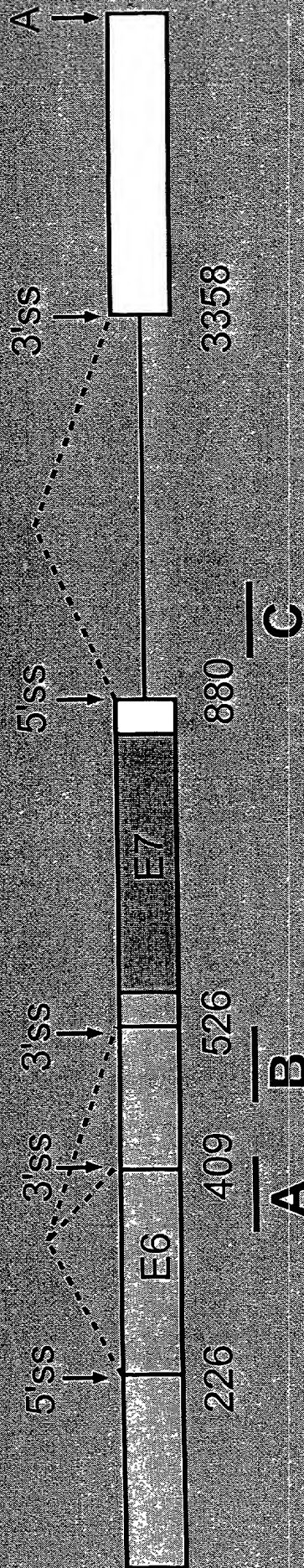
Note: Nucleotides in bold are modified to prevent PTMs cryptic splicing

FIGURE 54

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# Positions of HPV-PTM Targeting Domains

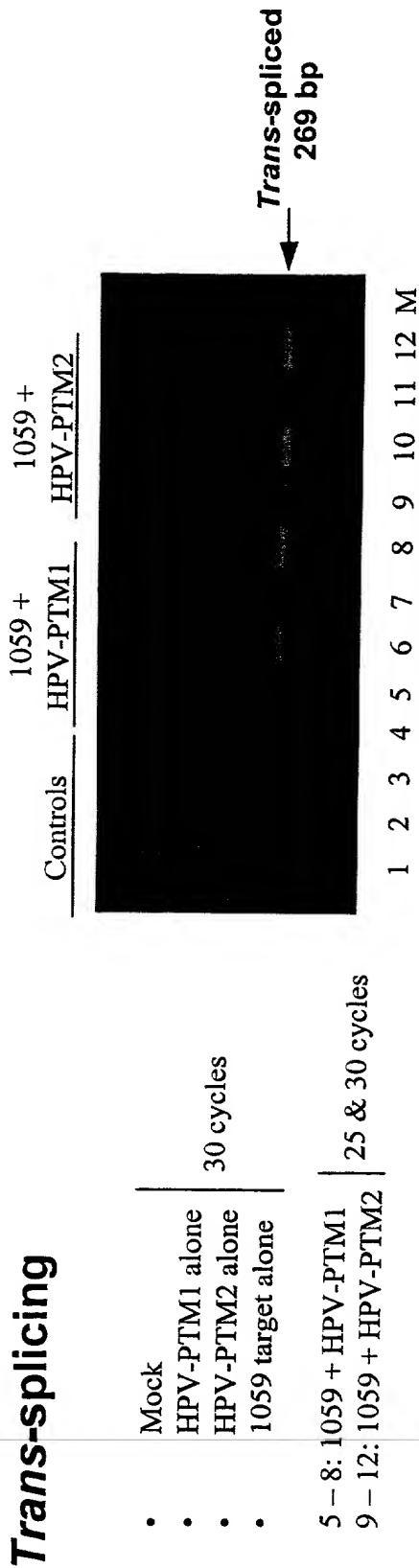
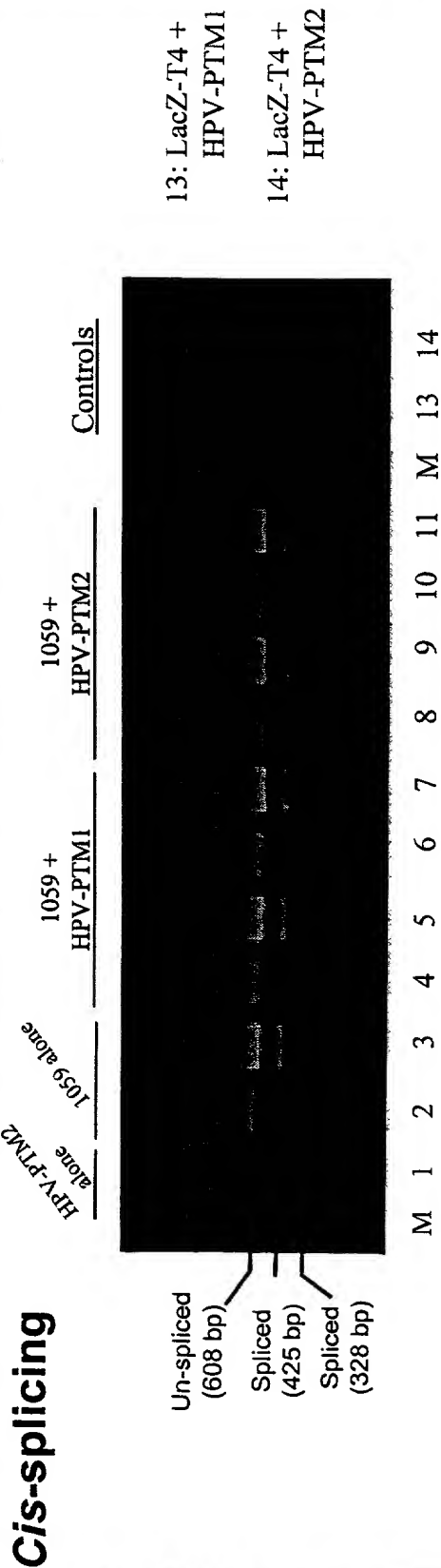


<u>Binding Domain</u>	
<u>PTM</u>	<u>Region</u>
HPV-PTM1	A
HPV-PTM2	A
HPV-PTM5	A+B
HPV-PTM6	A+B
HPV-PTM8	C
HPV-PTM9	C
	<u>Size (nt)</u>
	80
	149
	140
	117
	104
	174

INTRONN

FIGURE 55

# Trans-splicing Efficiency of HPV-PTMs in 293T Cells



RT-PCR Analysis of total RNA



# Trans-splicing between target pre-mRNA and PTM is accurate (293T cells)

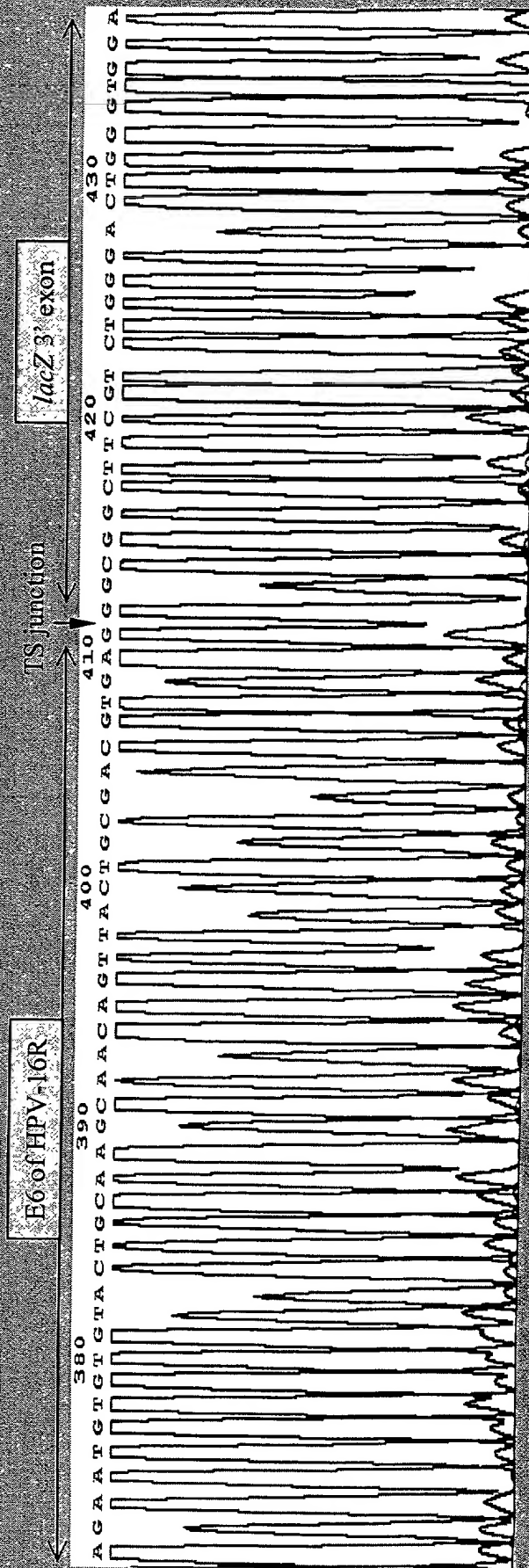
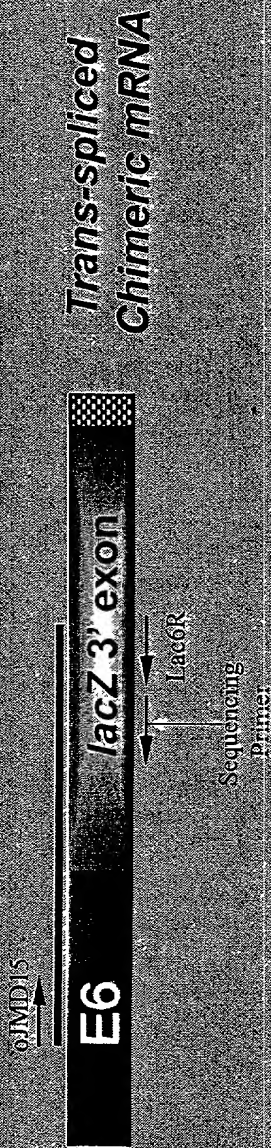
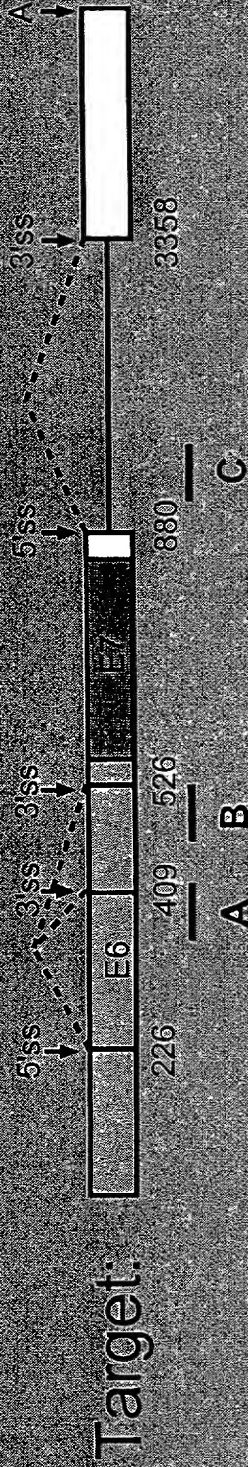


FIGURE 57

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# Trans-splicing in 293 Cells (Co-transfections)



PTM	<u>Binding Domain</u>		<u>% trans-spliced</u>	
	<u>Region</u>	<u>Size (nt)</u>	<u>226 sd</u>	<u>880 sd</u>
HPV-PTM1	A	80	69	0.6
HPV-PTM2	A	149	45	0.9
HPV-PTM5	A+B	140	55	0.8
HPV-PTM5ΔBP/PPT	A+B	140	0.5	0.2
HPV-PTM6	A+B	117	59	1
HPV-PTM8	C	104	7	37
HPV-PTM9	C	174	14	22
CF-PTM27	CF intron	411	0	0

Quantification of *trans*-splicing efficiency using real-time QRT-PCR

FIGURE 58



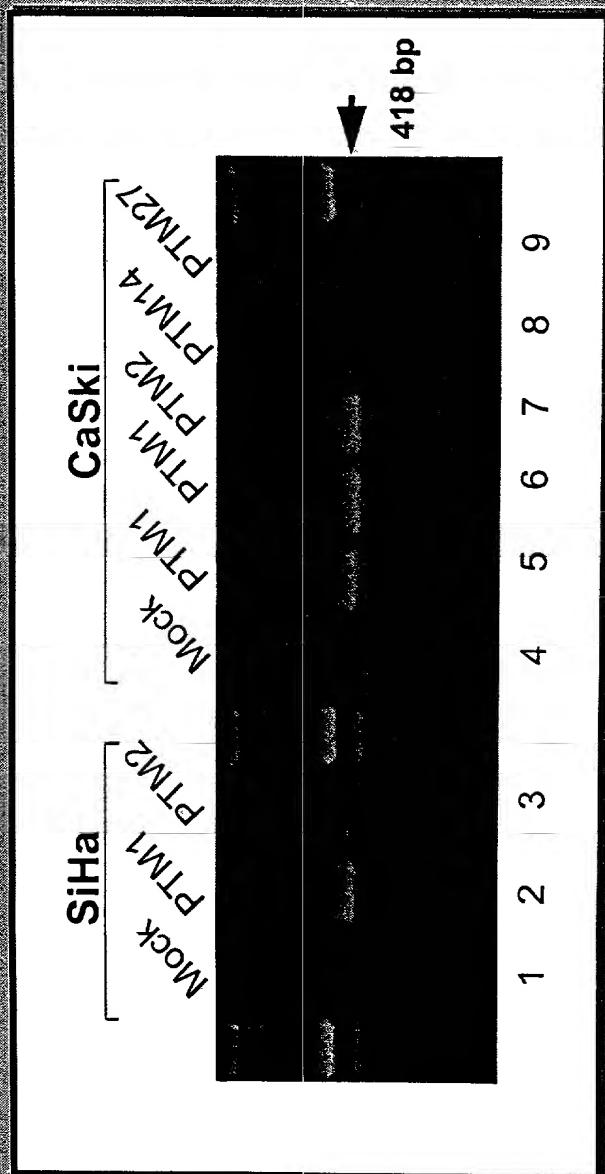
# Trans-splicing into Endogenous HPV Pre-mRNA Target in SiHa & CaSki Cells

## RT-PCR Conditions

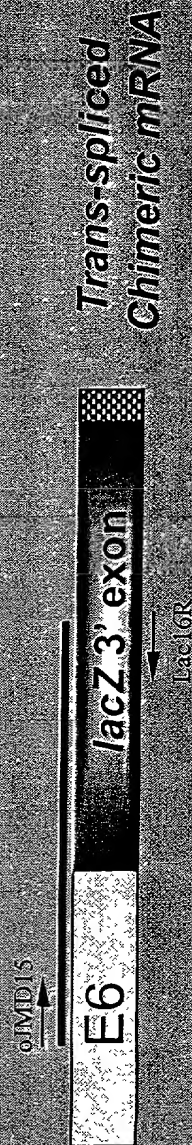
- Total RNA: 400 ng/rxn
- Primer's: oJMD15 + Lac16R
- # Cycles : 35
- Expected product : 418 bp

## Details

- PTM1, PTM2 : HPV targeted, specific
- PTM14 : CF targeted, non-specific, has 23 bp BD
- PTM14 : CF targeted, non-specific, has 411 bp BD



SiHa : Single copy  
CaSki : ~ 400-500 copy!!

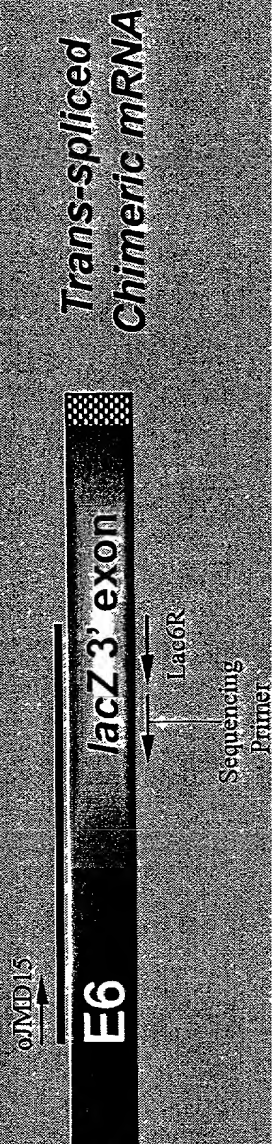


RT-PCR Analysis of total RNA

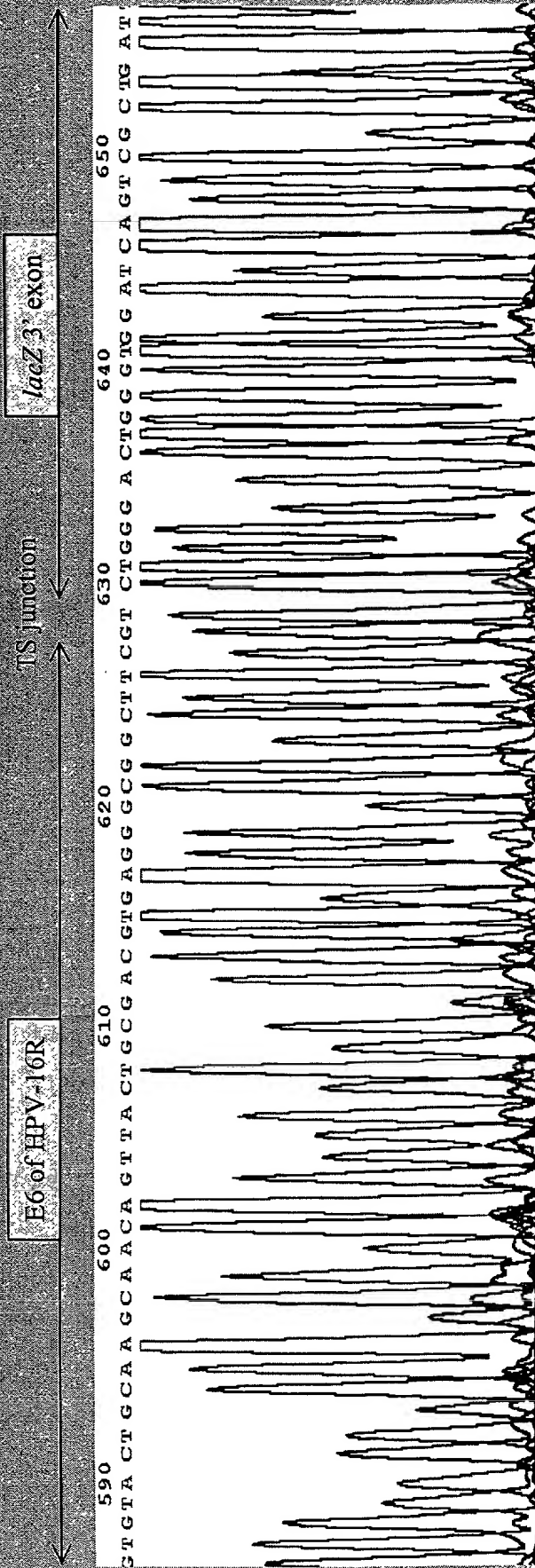
FIGURE 59

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# Accurate Trans-splicing of HPV-PTM1 in Si Ha Cells (Endogenous target pre-mRNA)



*Trans-spliced  
Chimeric mRNA*



INTRON

FIGURE 60

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# Trans-splicing in SiHa Transfections

(Endogenous target)

<u>PTM</u>	<u>% <i>trans</i>-spliced</u>
pcDNA3.1	0
HPV-PTM1	0.16
HPV-PTM5	0.12
HPV-PTM6	0.11
CF-PTM27	0

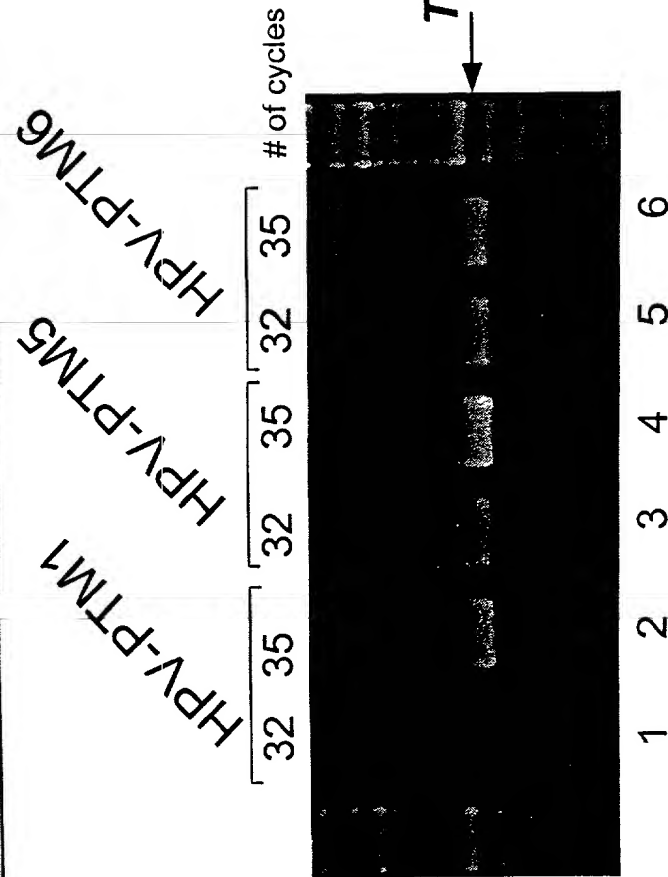
Quantification of *trans*-splicing efficiency using real-time QRT-PCR

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FIGURE 61

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# Trans-splicing Efficiency of HPV-PTM1, 5, & 6 in SiHa Cells



- SiHa cells transfected with 1.5 µg plasmid DNA, LipoPlus
- RNA isolated after 48 hr
- Total RNA: 500 ng/Rxn
- Primers: oJMD15 + Lac16R
- Expected product: 418 bp

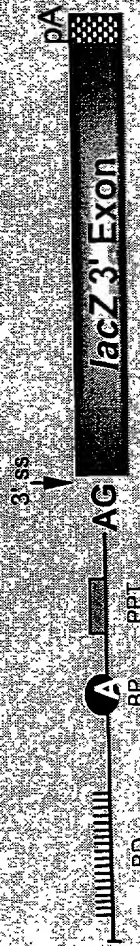
1, 3, 5, : 32 cycles  
2, 4, 6, : 35

FIGURE 62



# Deletion of polypyrimidine tract abolishes *trans*-splicing

HPV-PTM5 (has all the elements)



HPV-?PPT (PPT has been deleted)



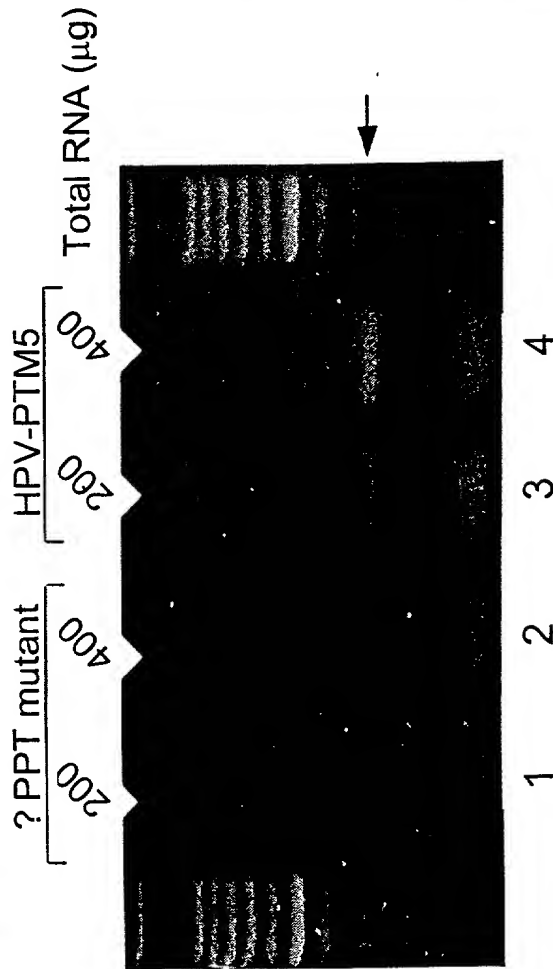
## Methods:

- SiHa cells transfected with 1.5  $\mu$ g of plasmid DNA
- Total RNA isolated after 48 hr and analyzed by RT-PCR (30 cycles)

Primers: oJMD15+Lac6R  
Expected product: 269 bp

Lanes 1 & 2: RNA from cells transfected with HPV-?PPT (mutant); No trans-splicing detected

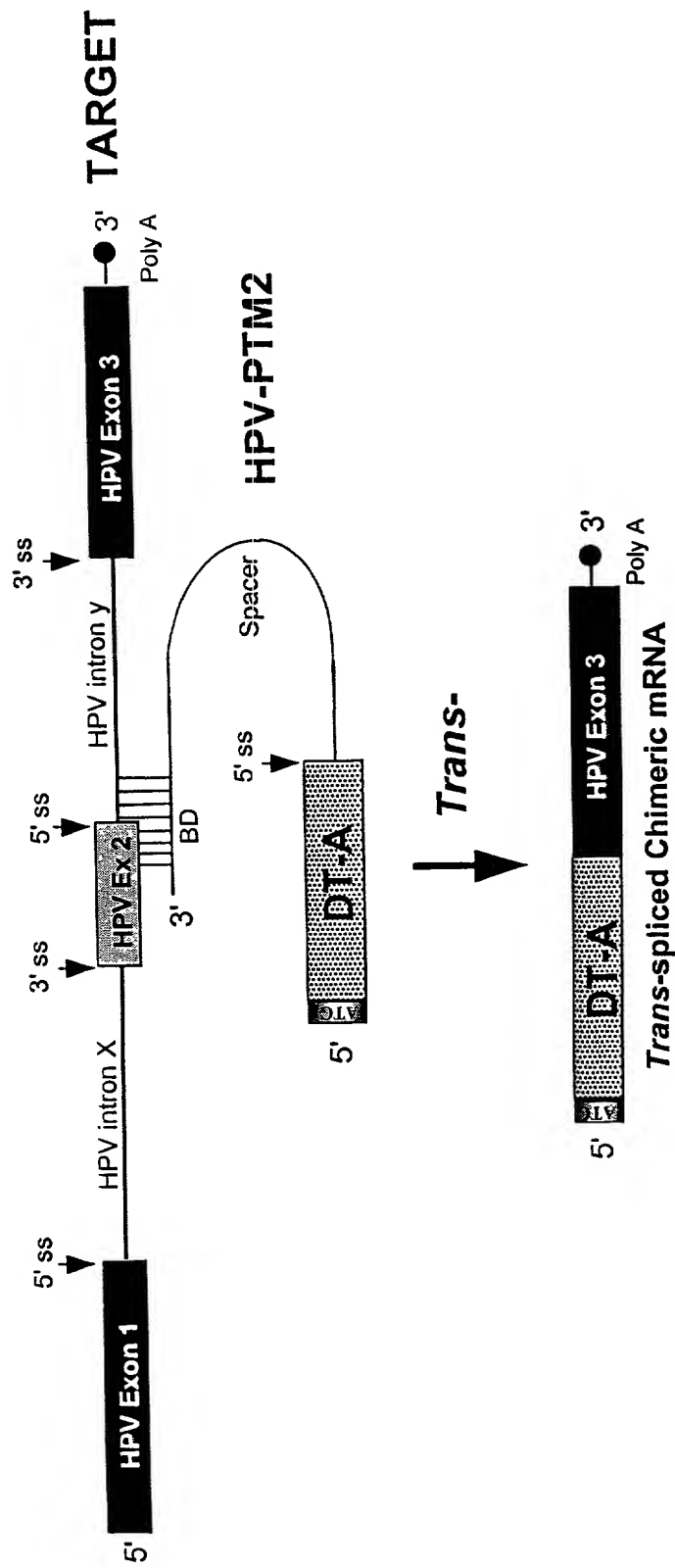
Lanes 3 & 4: RNA from cells transfected with HPV-PTM5 plasmid; trans-splicing Detected (269 bp product)



INTRONN

FIGURE 63

# SMaRT Strategy by 5' Exon Replacement



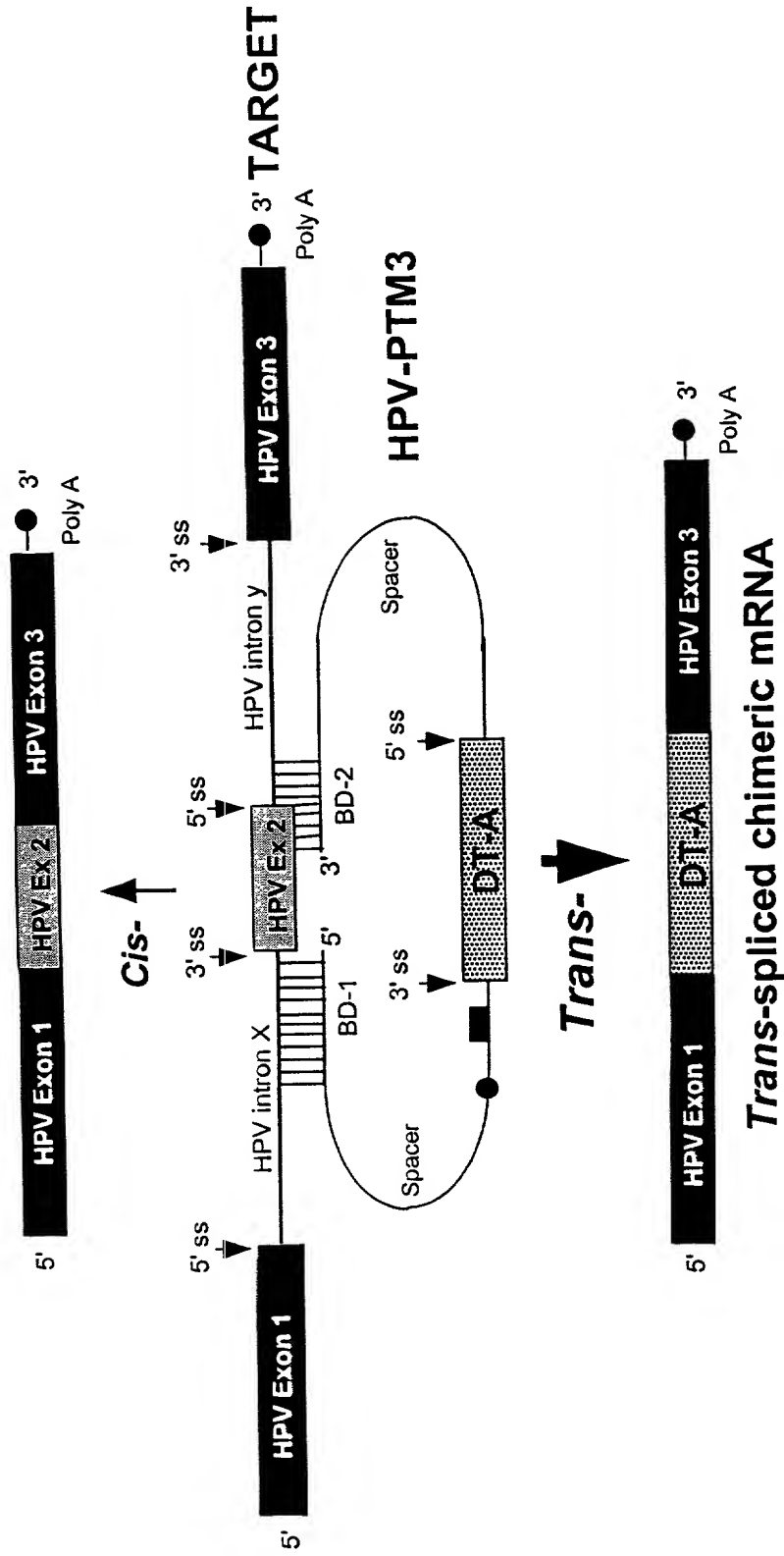
Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

INTRONN

FIGURE 64

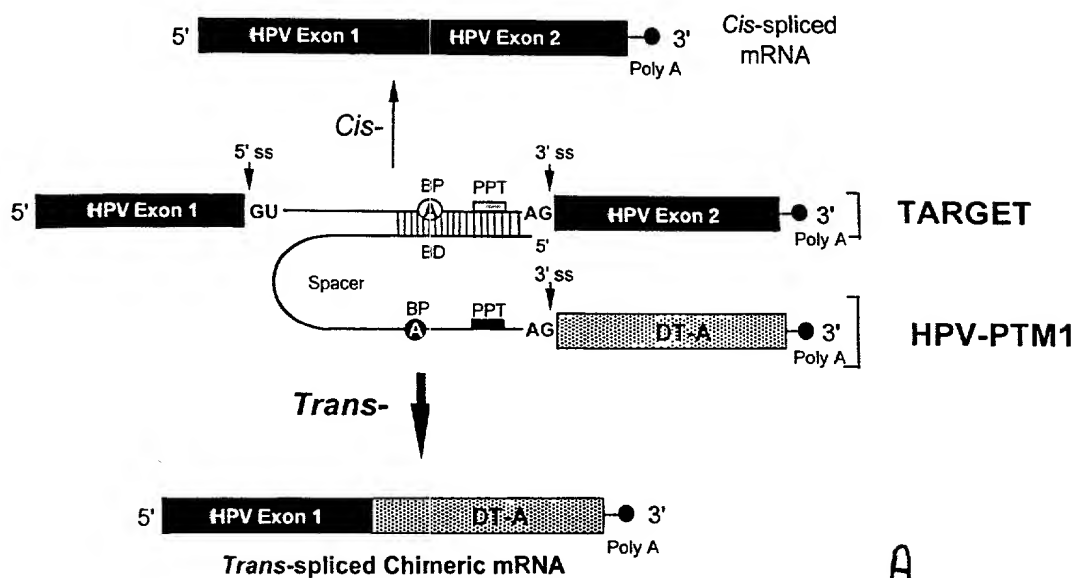
hp to 92

# Double *Trans*-splicing



Schematic diagram of a double *trans*-splicing PTM binding to the 3' and 5' splice sites of the HPV mini-gene target

**SMaRT Strategy by 3' Exon Replacement:** Schematic diagram of a PTM binding to the 3' splice site of the HPV mini-gene target



**SMaRT Strategy by 5' Exon Replacement:** Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

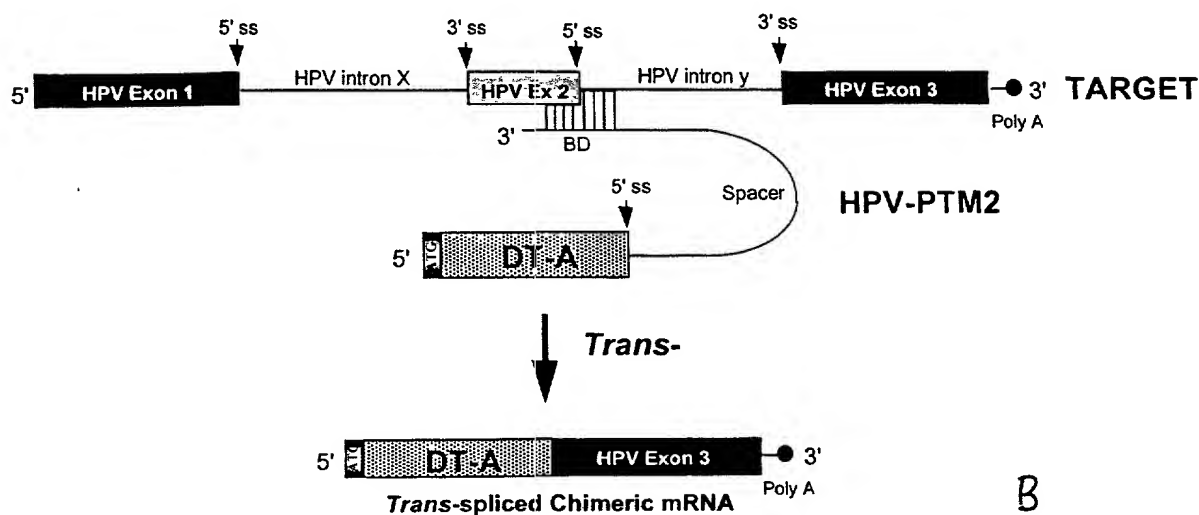


FIGURE 66

HPV-PTM3 (For Internal exon replacement)

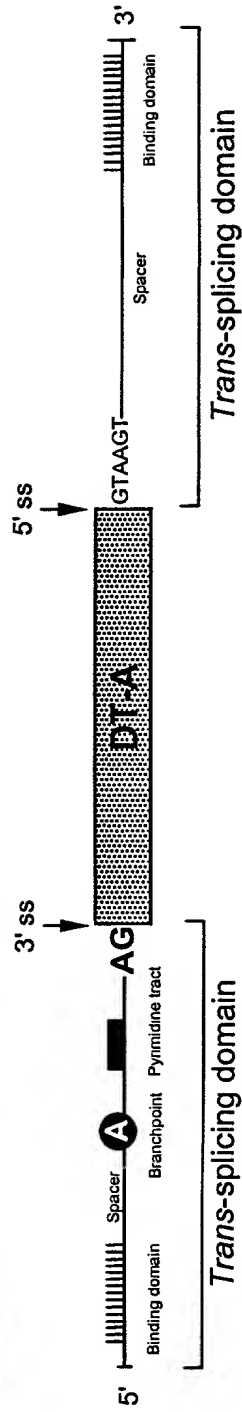


FIGURE 67